TENT COOPERATION TRE Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 May 2001 (09.05.01)	
International application No. PCT/NZ00/00174	**Applicant's or agent's file reference** 18134/8X109
International filing date (day/month/year) 04 September 2000 (04.09.00)	**Priority date** (day/month/year) 02 September 1999 (02.09.99)
Applicant GLARE, Travis, Robert et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   26 March 2001 (26.03.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
18134/8X109		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NZ 00/ 00174	04/09/2000	02/09/1999
Applicant		
NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTI...		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   NUCLEOTIDES SEQUENCES ENCODING AN INSECTIDAL PROTEIN COMPLEX FROM SERRATIA

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17

Present claim 17 relates to a ligand defined by reference to a desirable characteristic or property, namely binding to the polypeptide of claim 15.

The claim covers all products having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for such products.
In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved.
Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NZ 00/00174

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C12N15/70 C12N15/82 C07K14/24 C12Q1/68
A01N63/02 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, STRAND, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JACKSON T A ET AL: "PATHOGEN TO PRODUCT DEVELOPMENT OF SERRATIA-ENTOMOPHILA ENTEROBACTERIACEAE AS A COMMERCIAL BIOLOGICAL CONTROL AGENT FOR NEW ZEALAND GRASS GRUB COSTELYTRA-ZEALANDICA" JACKSON, T. A. AND T. R. GLARE (ED.). USE OF PATHOGENS IN SCARAB PEST, 1992, pages 191-198, XP000997900 0-946707-35-9. 1992 the whole document --- -/--	32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2001	06/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED [redacted] RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GRKOVIC STEVE ET AL: "Genes Essential for Amber Diseae in Grass Grubs Are Located on the Large Plasmid Found in Serratia entomophila and Serratia proteamaculans." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 61, no. 6, 1995, pages 2218-2223, XP000994573 ISSN: 0099-2240 cited in the application the whole document ---	
A	GLARE TRAVIS R ET AL: "Plasmid transfer among several members of the family Enterobacteriaceae increases the number of species capable of causing experimental amber disease in grass grub." FEMS MICROBIOLOGY LETTERS, vol. 139, no. 2-3, 1996, pages 117-120, XP000998482 ISSN: 0378-1097 cited in the application the whole document ---	
A	WO 99 42589 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); KRAMER VANCE CARY) 26 August 1999 (1999-08-26) the whole document ---	
A	WO 98 08932 A (DOW AGROSCIENCES LLC ;WISCONSIN ALUMNI RES FOUND (US)) 5 March 1998 (1998-03-05) the whole document ---	
A	WO 98 08388 A (MORGAN JAMES ALUN WYNNE ;JARRETT PAUL (GB); ELLIS DEBORAH JUNE (GB) 5 March 1998 (1998-03-05) the whole document ---	
A	WO 97 17432 A (WISCONSIN ALUMNI RES FOUND) 15 May 1997 (1997-05-15) the whole document ---	
A	BOWEN D ET AL: "INSECTICIDAL TOXINS FROM THE BACTERIUM Photorhabdus liminescens" SCIENCE,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,,US, vol. 280, 26 June 1998 (1998-06-26), pages 2129-2132, XP002115650 ISSN: 0036-8075 cited in the application ---	

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/NZ 00/00174

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	NUNEZ-VALDEZ M E ET AL: "The amb2 locus from Serratia entomophila confers anti-feeding effect on larvae of Costelytra zealandica (Coleoptera: Scarabaeidae)" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 172, no. 1, 12 June 1996 (1996-06-12), pages 75-79, XP004042712 ISSN: 0378-1119 cited in the application	
P,X	HURST MARK R H ET AL: "Plasmid-located pathogenicity determinants of Serratia entomophila, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of Photorhabdus luminescens." JOURNAL OF BACTERIOLOGY, vol. 182, no. 18, September 2000 (2000-09), pages 5127-5138, XP002166799 ISSN: 0021-9193 the whole document	1-4, 9-16, 21-27, 31,41

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NZ 00/00174

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9942589	A	26-08-1999	AU	3028699 A	06-09-1999
			EP	1054972 A	29-11-2000
WO 9808932	A	05-03-1998	AU	729228 B	25-01-2001
			AU	1050997 A	29-05-1997
			AU	2829997 A	19-03-1998
			BR	9606889 A	28-10-1997
			BR	9711441 A	24-10-2000
			CA	2209659 A	15-05-1997
			EP	0797659 A	01-10-1997
			EP	0970185 A	12-01-2000
			HU	9900768 A	28-06-1999
			JP	2000515024 T	14-11-2000
			PL	321212 A	24-11-1997
			PL	332033 A	16-08-1999
			SK	24699 A	10-04-2000
			SK	93197 A	06-05-1998
			TR	9901126 T	21-07-1999
			WO	9717432 A	15-05-1997
WO 9808388	A	05-03-1998	AU	4024997 A	19-03-1998
			BR	9711285 A	17-08-1999
			CN	1233938 A	03-11-1999
			EP	0923295 A	23-06-1999
			TR	9900435 T	21-06-1999
			ZA	9707373 A	15-02-1999
WO 9717432	A	15-05-1997	AU	729228 B	25-01-2001
			AU	1050997 A	29-05-1997
			BR	9606889 A	28-10-1997
			CA	2209659 A	15-05-1997
			EP	0797659 A	01-10-1997
			HU	9900768 A	28-06-1999
			PL	321212 A	24-11-1997
			PL	332033 A	16-08-1999
			SK	93197 A	06-05-1998
			AU	2829997 A	19-03-1998
			BR	9711441 A	24-10-2000
			EP	0970185 A	12-01-2000
			JP	2000515024 T	14-11-2000
			SK	24699 A	10-04-2000
			TR	9901126 T	21-07-1999
			WO	9808932 A	05-03-1998

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MES & WELLS
Private Bag 3140
HAMILTON
New Zealand

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *day/month/year*	1 DEC 2001
Applicant's or agent's file reference 18134/8X109SB	IMPORTANT NOTIFICATION

International Application No.	International Filing Date	Priority Date
PCT/NZ00/00174	4 September 2000	2 September 1999

Applicant

NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTELIMITED et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide

Name and mailing address of the IPEA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	GARETH COOK Telephone No. (02) 6283 2541

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 18134/8X109SB	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/NZ00/00174	International Filing Date *(day/month/year)* 4 September 2000	Priority Date *(day/month/year)* 2 September 1999

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] C12N 15/31, A01H 5/00, A01N 63/02, C07K 14/24, C12N 15/70, C12N 15/82, C12Q 1/68

Applicant

NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTELIMITED et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 5 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
26 March 2001	28 November 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer GARETH COOK Telephone No. (02) 6283 2541

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ00/00174

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed.

☒ the description, pages 1-2, 5-48, as originally filed,
pages , filed with the demand,
pages 3, 4, received on 5 October 2001 with the letter of 5 October 2001

☒ the claims, pages 52-54, as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages 49-51, received on 23 November 2001 with the letter of 23 November 2001

☒ the drawings, pages 1-8, as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the sequence listing part of the description:
pages 1-46, as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ00/00174

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims 1-41	YES
	Claims	NO
Inventive step (IS)	Claims 1-41	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-41	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

Novelty (N) and Inventive Step (IS) claims 1 to 41

The claims are to a an insecticidal protein complex, the polynucleotide encoding it and uses associated with protein and polypeptide. The closest prior art is considered to be Grkovic S *et al*, *Applied and Environmental Microbiology*, 1995, 61(6):2218-223 which discloses the plasmid which encodes for the insecticidal protein complex. The document however does not disclose the sequence of the polypeptides or their encoding polynucleotides, hence the claims are considered novel. It is also considered that it would require more than routine effort to identify the genes encoding the protein complex, hence the claims are considered to involve an inventive step. As such the claims meet the requirements of Articles 33(2) and 33(3) of the PCT.

Industrial Applicability (IA) claims 1 to 41

Claims 1 to 41 are considered to be Industrially Applicable under Article 33(4) of the PCT.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
SIMS, ALLEN, FINCH, LEWIS, MURPHY, ROGERS, TYRER-HARDING, WELLS,
29 Clarence Street
Private Bag 3140
Hamilton 2001, New Zealand
NEW ZEALAND

Applicant's or agent's file reference 18134/8X109	Date of mailing (day/month/year) 06/06/2001 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/NZ 00/ 00174	International filing date (day/month/year) 04/09/2000
Applicant NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTI...	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41–22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL–2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Mireille Claudepierre

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
18134/8X109		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NZ 00/ 00174	04/09/2000	02/09/1999
Applicant		
NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTI...		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

NUCLEOTIDES SEQUENCES ENCODING AN INSECTIDAL PROTEIN COMPLEX FROM SERRATIA

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 00/00174

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C12N15/70 C12N15/82 C07K14/24 C12Q1/68
A01N63/02 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, STRAND, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JACKSON T A ET AL: "PATHOGEN TO PRODUCT DEVELOPMENT OF SERRATIA-ENTOMOPHILA ENTEROBACTERIACEAE AS A COMMERCIAL BIOLOGICAL CONTROL AGENT FOR NEW ZEALAND GRASS GRUB COSTELYTRA-ZEALANDICA" JACKSON, T. A. AND T. R. GLARE (ED.). USE OF PATHOGENS IN SCARAB PEST, 1992, pages 191-198, XP000997900 0-946707-35-9. 1992 the whole document --- -/--	32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2001	06/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (July 1992)

INT ATIONAL SEARCH REPORT

International Application No
PC 00/00174

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRKOVIC STEVE ET AL: "Genes Essential for Amber Diseae in Grass Grubs Are Located on the Large Plasmid Found in Serratia entomophila and Serratia proteamaculans." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 61, no. 6, 1995, pages 2218-2223, XP000994573 ISSN: 0099-2240 cited in the application the whole document	
A	GLARE TRAVIS R ET AL: "Plasmid transfer among several members of the family Enterobacteriaceae increases the number of species capable of causing experimental amber disease in grass grub." FEMS MICROBIOLOGY LETTERS, vol. 139, no. 2-3, 1996, pages 117-120, XP000998482 ISSN: 0378-1097 cited in the application the whole document	
A	WO 99 42589 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); KRAMER VANCE CARY) 26 August 1999 (1999-08-26) the whole document	
A	WO 98 08932 A (DOW AGROSCIENCES LLC ;WISCONSIN ALUMNI RES FOUND (US)) 5 March 1998 (1998-03-05) the whole document	
A	WO 98 08388 A (MORGAN JAMES ALUN WYNNE ;JARRETT PAUL (GB); ELLIS DEBORAH JUNE (GB) 5 March 1998 (1998-03-05) the whole document	
A	WO 97 17432 A (WISCONSIN ALUMNI RES FOUND) 15 May 1997 (1997-05-15) the whole document	
A	BOWEN D ET AL: "INSECTICIDAL TOXINS FROM THE BACTERIUM Photorhabdus luminescens" SCIENCE,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,,US, vol. 280, 26 June 1998 (1998-06-26), pages 2129-2132, XP002115650 ISSN: 0036-8075 cited in the application	

-/--

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTE[redacted]TIONAL SEARCH REPORT

International Application No
PC[redacted] 00/00174

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NUNEZ-VALDEZ M E ET AL: "The amb2 locus from Serratia entomophila confers anti-feeding effect on larvae of Costelytra zealandica (Coleoptera: Scarabaeidae)" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 172, no. 1, 12 June 1996 (1996-06-12), pages 75-79, XP004042712 ISSN: 0378-1119 cited in the application	
P,X	HURST MARK R H ET AL: "Plasmid-located pathogenicity determinants of Serratia entomophila, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of Photorhabdus luminescens." JOURNAL OF BACTERIOLOGY, vol. 182, no. 18, September 2000 (2000-09), pages 5127-5138, XP002166799 ISSN: 0021-9193 the whole document	1-4, 9-16, 21-27, 31,41

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17

Present claim 17 relates to a ligand defined by reference to a desirable characteristic or property, namely binding to the polypeptide of claim 15.

The claim covers all products having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for such products.
In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved.
Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ 00/00174

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 17
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NZ 00/00174

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9942589 A	26-08-1999	AU 3028699 A	06-09-1999
		EP 1054972 A	29-11-2000
WO 9808932 A	05-03-1998	AU 729228 B	25-01-2001
		AU 1050997 A	29-05-1997
		AU 2829997 A	19-03-1998
		BR 9606889 A	28-10-1997
		BR 9711441 A	24-10-2000
		CA 2209659 A	15-05-1997
		EP 0797659 A	01-10-1997
		EP 0970185 A	12-01-2000
		HU 9900768 A	28-06-1999
		JP 2000515024 T	14-11-2000
		PL 321212 A	24-11-1997
		PL 332033 A	16-08-1999
		SK 24699 A	10-04-2000
		SK 93197 A	06-05-1998
		TR 9901126 T	21-07-1999
		WO 9717432 A	15-05-1997
WO 9808388 A	05-03-1998	AU 4024997 A	19-03-1998
		BR 9711285 A	17-08-1999
		CN 1233938 A	03-11-1999
		EP 0923295 A	23-06-1999
		TR 9900435 T	21-06-1999
		ZA 9707373 A	15-02-1999
WO 9717432 A	15-05-1997	AU 729228 B	25-01-2001
		AU 1050997 A	29-05-1997
		BR 9606889 A	28-10-1997
		CA 2209659 A	15-05-1997
		EP 0797659 A	01-10-1997
		HU 9900768 A	28-06-1999
		PL 321212 A	24-11-1997
		PL 332033 A	16-08-1999
		SK 93197 A	06-05-1998
		AU 2829997 A	19-03-1998
		BR 9711441 A	24-10-2000
		EP 0970185 A	12-01-2000
		JP 2000515024 T	14-11-2000
		SK 24699 A	10-04-2000
		TR 9901126 T	21-07-1999
		WO 9808932 A	05-03-1998

PATENT COOPERATION TREAT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 18134/8X109SB	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/NZ00/00174**	International Filing Date *(day/month/year)* 4 September 2000	Priority Date *(day/month/year)* 2 September 1999
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C12N 15/31, A01H 5/00, A01N 63/02, C07K 14/24, C12N 15/70, C12N 15/82, C12Q 1/68		
Applicant NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTELIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **5** sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand 26 March 2001	Date of completion of the report 28 November 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **GARETH COOK** Telephone No. (02) 6283 2541

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ00/00174

I. Basis of the report

1. With regard to the **elements** of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-2, 5-48**, as originally filed,
  pages , filed with the demand,
  pages **3, 4**, received on **5 October 2001** with the letter of **5 October 2001**
- [X] the claims, pages **52-54,** as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **49-51,** received on **23 November 2001** with the letter of **23 November 2001**
- [X] the drawings, pages **1-8,** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the sequence listing part of the description:
  pages **1-46,** as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [X] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [X] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [X] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **1-41**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-41**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-41**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

Novelty (N) and Inventive Step (IS) claims 1 to 41

The claims are to a an insecticidal protein complex, the polynucleotide encoding it and uses associated with protein and polypeptide. The closest prior art is considered to be Grkovic S *et al*, *Applied and Environmental Microbiology*, 1995, 61(6):2218-223 which discloses the plasmid which encodes for the insecticidal protein complex. The document however does not disclose the sequence of the polypeptides or their encoding polynucleotides, hence the claims are considered novel. It is also considered that it would require more than routine effort to identify the genes encoding the protein complex, hence the claims are considered to involve an inventive step. As such the claims meet the requirements of Articles 33(2) and 33(3) of the PCT.

Industrial Applicability (IA) claims 1 to 41

Claims 1 to 41 are considered to be Industrially Applicable under Article 33(4) of the PCT.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16305 A2

(51) International Patent Classification[7]: C12N 15/00

(21) International Application Number: PCT/NZ00/00174

(22) International Filing Date:
4 September 2000 (04.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
337610 2 September 1999 (02.09.1999) NZ

(71) Applicant *(for all designated States except US)*: **NEW ZEALAND PASTORAL AGRICULTURE RESEARCH INSTITUTE LIMITED** [NZ/NZ]; 5th floor, Tower Block, Ruakura Research Centre, East Street, Hamilton 2001 (NZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GLARE, Travis, Robert** [AU/NZ]; 38 Whincorps Road, Halswell, Christchurch 8003 (NZ). **HURST, Mark, Robin, Holmes** [NZ/NZ]; 148 Hendersons Road, Hoon Hay, Christchurch 8002 (NZ). **JACKSON, Trevor, Anthony** [NZ/NZ]; 407 Halswell Road, Halswell, Christchurch 8003 (NZ).

(74) Agents: **SIMS, Anthony, W.** et al.; 29 Clarence Street, Private Bag 3140, Hamilton 2001 (NZ).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/16305 A2

(54) Title: NUCLEOTIDE SEQUENCES

(57) Abstract: The present invention concerns novel nucleotide sequences encoding proteins from the Enterobacteriaceae, *Serratia entomophila* and *Serratia proteamaculans*, and the use of said nucleotide sequences and proteins for inherent insecticidal and potentially metazoacidal properties. The invention relates to an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with the nucleic acid molecule under standard hybridisation conditions. The nucleotide sequences include a pathogenicity-encoding region cloned from bacteria *Serratia entomophilia* and *S. proteamaculans*. The region contain pathogenic determinants of a disease that affect the grass grub, *Costelytra zealandica Coleoptera: Scarabaeidae*, an important insect pasture pest in New Zealand. The proteins encoded by determined genes may be used for insect control whether as an inundative pesticide, within baits or expressed in other organisms such as plants or microbes.

10/070489
PCT/NZ00/00174
JC13 Rec'd PCT/PTO 0 1 MAR 2002

NUCLEOTIDE SEQUENCES

TECHNICAL FIELD

The present invention concerns novel nucleotide sequences encoding insecticidal proteins from the Enterobacteriaceae, *Serratia entomophila* and *Serratia proteamaculans,* and the use of said nucleotide sequences and insecticidal proteins.

BACKGROUND ART

Some *Serratia entomophila* and *Serratia proteamaculans* strains in New Zealand are known to cause a disease in the major scarab pest, *Costelytra zealandica* (New Zealand grass grub). The disease was first discovered and described by Trought and Jackson (1982) and was later named amber disease after the distinctive colour of affected insects (Stucki et al. 1984). One species capable of causing the disease, *Serratia entomophila,* was developed into a commercially-available product ("Invade") in 1989.

The disease is highly host specific, only know to infect a single indigenous species of New Zealand scarab larva. The disease appears unique among insects and results not from rapid invasion of the haemocoel, but from a slow colonisation of the gut. The disease has a distinct phenotypic progression, with infected hosts ceasing feeding within 2-5 days of ingesting pathogenic cells. The normally black gut clears around this time (Jackson et al. 1993) and the levels of the major gut digestive enzymes (trypsin and so forth) decreases sharply (Jackson, 1995). The clearance of the gut results in a characteristic amber colour of the infected hosts. The larvae may remain in this state for a prolonged period (1-3 months) before bacteria eventually invade the haemocoel, causing rapid death.

The finding of a plasmid that apparently encoded the disease was reported in Glare et al. (1993) by showing a correlation between pADAP presence and disease occurrence in

bacterial strains. This was further confirmed by Glare et al. (1996) who showed that transfer of the plasmid from pathogenic to non-pathogenic strains resulted in a change to pathogenic.

Grkovic et al. (1995) showed that disruption of the plasmid by transposon insertion could alter pathogenicity without fully defining the area containing the gene cassette. By marker exchange, they showed that a 10.5kb *Hin*dIII (pGLA20) construct from pADAP encoded some functions of amber disease. However, the clone did not contain all disease encoding plasmid-borne regions.

Another region involved in amber disease encoding was located by Nunez-Valdez and Mahanty (1996). They located a locus, *amb2,* by transposon mutagensis and searching a cosmid genomic library. This region was chromosomally located and was involved in antifeeding in the larvae of *Costelytra zealandica.* However, the current applicant's research has demonstrated that the *amb2* region is located on pADAP remote from the virulence gene and is probably regulatory in function.

Insecticidal toxins which share some protein homology to the *Serratia* insecticidal proteins of the present invention have been recently discovered (PCT/US96/18803; PCT/US97/07657) by a group at Wisconsin University (Blackburn et al. 1998; Bowen et al. 1998; Bowen and Ensign 1998). These insecticidal toxins are produced from a gene region in *Photorhabdus luminescens* which resembles the *Serratia* virulence region in the clustering of the genes and at the protein level, but has very little DNA homology with the *Serratia* genes. They have shown high molecular weight proteins from *Photorhabdus luminescens* are insecticidal to a number of insects from different orders. The lack of DNA homology over the majority of the region, as opposed to protein homology, between the *Serratia* genes and *Photorhabdus* genes suggests that these proteins have evolved as a result of convergent evolution leading to the formation of a distinct protein family with a

common function.

The present applicant has now found that three regions of the pADAP plasmid are required for full insecticidal function. Sequence analysis of these three regions has shown that the present applicant has isolated and identified a novel toxin from *Serratia* species that belongs to a new family of insecticidal toxins. It is broadly to this toxin that the present invention is directed.

DISCLOSURE OF INVENTION

According to a first aspect of the present invention, there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 1 which encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof which have at least 75% nucleic acid homology to SEQ ID NO: 1 and are capable of hybridising with said nucleic acid molecule under stringent hybridisation conditions.

The invention also provides an isolated nucleic acid molecule comprising the nucleotide sequence 1995-18937 of SEQ ID NO: 1 which encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with said nucleic acid molecule under standard hybridisation conditions.

The invention also provides an isolated nucleic acid molecule comprising one or more of the nucleotide sequences 2411-9547, 9589-13883 or 14546-17467 of SEQ ID NO: 1 which encode insecticidal proteins, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with said nucleic acid molecule under standard hybridisation conditions.

Preferably the nucleic acid molecule comprises all of nucleotide sequences 2411-9547, 9598-13884 and 14546-17467 of SEQ ID NO: 1.

The invention further relates to an isolated nucleic acid molecule comprising a sequence of SEQ ID NO: 1, nucleotides 1955-18937 of SEQ ID NO: 1 or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein. For example, the at least one further nucleotide sequence may be the nucleotide sequence which codes for the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins and so forth.

The nucleic acid molecule may comprise DNA, cDNA or RNA.

Preferably said fragment, neutral mutation or homolog thereof is capable of hybridising to said nucleic acid molecule under stringent hybridisation conditions.

The invention further relates to nucleic acid molecules which hybridise to the nucleotide sequence of SEQ ID NO: 1, or nucleotides 1955-18937, 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1 if there is at least 75% or greater identity between the sequences.

The nucleic acid molecule may be isolated from *Serratia entomophila* or *Serratia proteamaculans* strains.

Also provided by the present invention are recombinant expression vectors containing the nucleic acid molecule of the invention and hosts transformed with the vector of the invention capable of expressing a polypeptide of the invention.

The vector may be selected from any suitable natural or artificial plasmid/vector. For example, pUC 19 (Yannish-Perron et al. 1995), pProEX HT (GibcoBRL, Gaithersburg, MD, USA), pBR322 (Bolivar et al. 1977), pACYC184 (Chang et al. 1978), pLAFR3 (Staskowicz et al. 1987), and so forth.

In a further aspect, the invention provides a method of producing a polypeptide of the invention comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with a vector as defined above to express the encoded polypeptide or peptide; and

(b) recovering the expressed polypeptide or peptide.

An additional aspect of the present invention provides a ligand that binds to a polypeptide of the invention. Most usually, the ligand is an antibody or antibody binding fragment. Such ligands also form a part of this invention.

According to a further aspect of the present invention there are provided probes and primers comprising a fragment of the nucleic acid molecule of the invention capable of hybridising under stringent conditions to a native insecticidal gene sequence. Such probes and primers are useful, for example, in studying the structure and function of this novel gene and for obtaining homologs of the gene from bacteria other than *Serratia* sp.

According to a still further aspect of the present invention there is provided a polypeptide having insecticidal activity encoded by the nucleic acid molecule of the invention, or a functional fragment, neutral mutation or homolog thereof.

The polypeptide may comprise the amino acid sequence of SEQ ID NO: 1 or a functional fragment, neutral mutation or homolog thereof.

The polypeptide may comprise amino acids 32-5118 of SEQ ID NO: 1.

The polypeptide may comprise at least one amino acid sequence of SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5 or SEQ ID NO: 6.

Preferably the polypeptide comprises amino acid sequence SEQ ID NO: 4; SEQ ID NO: 5

and SEQ ID NO: 6.

More preferably the polypeptide comprises all of SEQ ID NOs: 2-6.

Conveniently, the polypeptide of the invention is obtained by expression of a DNA sequence coding therefore in a host cell or organism.

The polypeptide may comprise the amino acid sequence of SEQ ID NO: 1 linked to at least one further amino acid sequence encoding an insecticidal protein. For example, the at least one further amino acid sequence may be the amino acid sequence which codes for *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescents* toxins etc.

The invention further relates to polypeptides comprising at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity to SEQ ID NO: 1.

The polypeptide may be produced by expression of a vector comprising the nucleic acid molecule of the invention or a functional fragment, neutral mutation or homolog thereof, in a suitable host cell.

According to a further aspect, there is provided an insecticidal composition comprising at least the polypeptide of the invention and an agriculturally acceptable carrier such as would be known to a person skilled in the art. More than one polypeptide of the invention can of course, be included in the composition. In addition, the composition may comprise one or more additional pesticides, for example, compounds known to possess herbicidal, fungicidal, insecticidal or nematicidal activity.

The composition may further comprise other known insecticidally active agents, such as *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescents* toxins

and so forth.

According to a further aspect, there is provided a method of combating pests, especially insects at a locus or host for the pest infested with or liable to be infested therewith, said method comprising applying to a locus, host and/or the pest, an effective amount of the polypeptide of the invention that has functional insecticidal activity against said pest.

According to a further aspect the invention provides a method of inducing amber disease or like condition in insects comprising delivery to an insect an effective amount of the polypeptide of the invention that has functional insecticidal activity against said insect.

The insect may be selected from the order comprising Coleoptera (such as the black beetle, *Heteronychus arator* (F.), or the black vine weevil, *Otiorhynchus sulcatus* (F.)); Dictyoptera (eg. The German cockroach, *Blattella germanica* (L.), or the subterranean termite *Coptotermes* spp,); Diptera (eg. the housefly *Musca domestica* L. or the blowfly *Lucillia cuprina* (Wiedermann); Orthoptera (eg. The black field cricket *Telleogryllus commodus* (Walker) or the migratory locust *Locusta migratoria* L.); Hymenoptera (eg. The German wasp, *Vespula germanica* F.)); Hemiptera (such as the green vegetable bug *Nezara viridula* (L.) or the green peach aphid *Myzus persicae* (Sulzer)) the Lepidoptera (eg. the tomato fruitworm, *Helicoverpa armigera* (Walker), or the codling moth, *Laspeyresia pomonella* (L.)).

The insecticidal polypeptide may be delivered to the insect orally either as a solid bait matrix, as a sprayable insecticide sprayed onto a substrate upon which the insect feeds, applied directly to the soil subsurface or as a drench or is expressed in an transgenic plant, bacterium, virus or fungus upon which the insect feeds, or by any other suitable method which would be obvious to a person skilled in the art.

According to a further aspect, the invention provides a transgenic plant, bacterium virus or

fungus, incorporating in its genome, a nucleic acid molecule of the invention providing the plant, bacterium virus or fungus with an ability to express an effective amount of an insecticidal polypeptide.

DEFINITIONS AND METHODS

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention.

Definitions of common terms in molecular biology may also be found in Lewin, *Genes V*, Oxford University Press: New York, 1994.

The term "native" refers to a naturally-occurring nucleic acid or polypeptide, including, wild-type sequence and alleles thereof.

A "homolog" has at least one of the biological activities of the nucleic acid or polypeptide of the invention and comprises at least 50-70% identical amino acid or nucleic acid sequence thereto, preferably 75-85% and most preferably 90-95% identical amino acid or nucleic acid sequence thereto.

The term "neutral mutation" means a mutation, (that is - a change in the nucleotide or polypeptide sequence such as by deletion, substitution, inversion or insertion, any of which have no effect on the function of the encoded protein).

As indicated above, also possible are variants of the polypeptide or peptide that differ from the native amino acid sequence by insertion, substitution or deletion of one or more amino acids. Where such a variant is desired, the nucleotide sequence of the native DNA is altered appropriately. This alteration can be made through elective synthesis of the DNA, or by modification of the native DNA by, for example, site specific or cassette mutagenesis. Preferably, where portions of cDNA or genomic DNA require sequence modifications, site-

specific primer directed mutagenesis is employed using techniques standard in the art.

In a further aspect, the present invention consists in replicable transfer vector suitable for use in preparing a polypeptide of the invention. These vectors may be constructed according to techniques well known in the art, or may be selected from cloning vecotrs available in the art.

The cloning vector may be selected according to the host or host cell to be used. Useful vectors will generally have the following characteristics:

(a) the ability to self-replicate;

(b) the possession of a single target for any particular restriction endonuclease; and

(c) desirably, carry genes for a readily selectable marker such as antibiotic resistance.

Two major types of vector possessing these characteristics are plasmids and bacterial viruses (bacteriophages or phages). Presently preferred vectors include plasmids pMOS-Blue, pGem-T and pUC8.

The nucleic acids of the present invention can be free in solution, or attached by conventional means to a solid support, or present in an expression vector or any other type of plasmid.

The term "isolated" means substantially separated or purified away from contaminating sequences in the cell or organism in which the nucleic acid naturally occurs and includes nucleic acids purified by standard purification techniques as well as nucleic acids prepared by recombinant technology and those chemically synthesised.

The terms "DNA construct" means a construct incorporating the nucleic acid molecule of the present invention, or a fractional fragment, neutral mutation or homolog thereof in a

position whereby the protein coding sequence is under the control of an operably linked promoter capable of expression in a plant cell. Such promoters are well known in the art.

A fragment of a nucleic acid molecule according to the present invention is a portion of the nucleic acid that is less than full length and comprises at least a minimum length capable of hybridising specifically with a nucleic acid molecule according to the present invention (or a sequence complementary thereto) under stringent conditions as defined below. A fragment according to the present invention has at least one of the biological activities of the nucleic acid or polypeptide of the present invention.

Nucleic acid probes and primers can be prepared based on nucleic acids according to the present invention (for example, the sequence of SEQ ID NO: 1). A "probe" comprises an isolated nucleic acid attached to a detectable label or reporter molecule well known in the art. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, preferably DNA oligonucleotides 15 nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridisation to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, preferably a DNA polymerase. Primer pairs can be used for amplification of a nucleic acid sequence, (for example, by the polymerase chain reaction (PCR) or other nucleic acid amplification methods well known in the art). PCT-primer pairs can be derived from the sequence of a nucleic acid according to the present invention, (for example, by using computer programs intended for that purpose such as Primer (Version 0.5© 1991, Whitehead Institute for Biomedical Research, Cambridge, MA)).

Methods for preparing and using probes and primers are described, for example, in Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed, vol. 1-3, ed Sambrook

et al. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY, 1989.

Probes or primers can be free in solution or covalently or noncovalently attached to a solid support by standard means.

The term "operably linked" means a first nucleic acid sequence linked to a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in reading frame.

The DNA molecules of the invention may be expressed by placing them in operable linkage with suitable control sequences in a replicable expression vector. Control sequences may include origins of replication, a promoter, enhancer and transcriptional terminator sequences, amongst others. The selection of the control sequence to be included in the expression vector is dependent on the type of host or host cell intended to be used for expressing the DNA.

A "recombinant" nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids (for example, by genetic engineering techniques).

Techniques for nucleic acid manipulation are described generally in, for example, Sambrook et al. (1989).

Large amounts of a nucleic acid according to the present invention can be produced by recombinant means well known in the art or by chemical synthesis.

Natural or synthetic nucleic acids according to the present invention can be incorporated into recombinant nucleic acid constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Usually the DNA constructs will be suitable for replication in a unicellular host, such as *E. coli* or other commonly used bacteria, but can also be introduced into yeast, mammalian, plant or other eukaryotic cells.

Preferably, such a nucleic acid construct is a vector comprising a replication system recognised by the host. For the practice of the present invention, well known compositions and techniques for preparing and using vectors, host cells, introduction of vectors into host cells and so forth., are employed, as discussed, *inter alia,* in Sambrook et al (1989).

A cell, tissue, organ, or organism into which has been introduced a foreign nucleic acid, such as a recombinant vector, is considered "transformed" or "transgenic". The DNA construct comprising a DNA sequence according to the present invention that is present in a transgenic host cell, particularly a transgenic plant, is referred to as a "transgene". The term "transgenic" or "transformed" when referring to a cell or organism, also includes;

(1) progeny of the cell or organism, and

(2) plants produced from a breeding program employing such a "transgenic" plant as a parent in a cross and exhibiting an altered phenotype resulting from the presence of the recombinant DNA construct.

Generally, procaryotic, yeast, insect, or mammalian cells are useful hosts. Also included within the term hosts are plasmid vectors. Suitable procaryotic hosts include *E. coli, Bacillus* species and various species of *Pseudomonas*. Commonly used promoters such as β-lactamase (penicillinase) and lactose (lac) promoter systems are all well known in the art. Any available promoter system compatible with the host of choice can be used. Vectors used in yeast are also available and well known. A suitable example is the 2 micron origin

of replication plasmid.

Similarly, vectors for use in mammalian cells are also well known. Such vectors include well known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences, *Herpes simplex* virus, and vectors derived from a combination of plasmid and phage DNA.

Further eucaryotic expression vectors are known in the art (for example in P.J. Southern and P. Berg, *J. Mol. Appl. Genet. 1* 327-341 (1982); S. Subramani et al., *Mol. Cell. Biol. 1,* 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "*Amplification and Expression of Sequences Cotransfected with a Modular Dihydrofolate Reducase Complementary DNA Gene, J. Mol. Biol. 159,* 601-621 (1982); R.J. Kaufmann and P.A. Sharp, *Mol. Cell. Biol. 159,* 601-664 (1982); S.I. Scahill et al., "*Expressions and Characterisation of the Product of a Human Immune Interferon DNA Gene in Chinese Hamster Ovary Cells,*" *Proc. Natl. Acad. Sci. USA. 80,* 4654-4659 (1983); G. Urlaub and L.A. Chasin, *Proc. Natl. Acad. Sci. USA. 77,* 4216-4220, (1980).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the glycolytic promoters of yeast acid phosphatase, (for example, Pho5), the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus (for example, the early and late promoters of SV-40), and other sequences known to control the expression of genes of prokaryotic and eucaryotic cells and their viruses or combinations thereof.

In the construction of a vector it is also an advantage to be able to distinguish the vector incorporating the foreign DNA from unmodified vectors by a convenient and rapid assay.

Reporter systems useful in such assays include reported genes, and other detectable labels which produce measurable colour changes, antibiotic resistance and the like. In one preferred vector, the β-galactosidase reporter gene is used, which gene is detectable by clones exhibiting a blue phenotype on X-gal plates. This facilitates selection. In one embodiment, the β-galactosidase gene may be replaced by a polyhedrin-encoding gene; which gene is detectable by clones exhibiting a white phenotype when stained with X-gal.

This blue-white colour selection can serve as a useful marker for detecting recombinant vectors.

Once selected, the vectors may be isolated from the culture using routine procedures such as freeze-thaw extraction followed by purification.

For expression, vectors containing the DNA of the invention to be expressed and control signals are inserted or transformed into a host or host cell. Some useful expression host cells include well-known prokaryotic and eucaryotic cells. Some suitable prokaryotic hosts include, for example, *E. coli*, such as *E. coli*, S G-936, *E. coli* HB 101, *E. coli* W3110, *E. coli* X1776, *E. coli*, X2282, *E. coli* DHT and *E. coli* MR01, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis* and *Streptomyces*. Suitable eucaryotic cells include yeast and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

Depending on the host used, transformation is performed according to standard techniques appropriate to such cells. For prokaryotes or other cells that contain substantial cell walls, the calcium treatment process (Cohen, S N *Proceedings, National Academy of Science, USA* 69 2110 (1972)) may be employed. For mammalian cells without such cell walls the calcium phosphate precipitation method of Graeme and Van Der Eb, *Virology* 52:546 (1978) is preferred. Transformations into plants may be carried out using *Agrobacterium tumefaciens* (Shaw et al., Gene 23:315 (1983)) or into yeast according to the method of Van

Solingen et al. *J. Bact.* 130:946 (1977) and Hsiao et al. *Proceedings, National Academy of Science,* 76:3829 (1979).

Upon transformation of the selected host with an appropriate vector the polypeptide, or peptide encoded can be produced, often in the form of fusion protein, by culturing the host cells. The polypeptide, or peptide, of the invention may be detected by rapid assays as indicated above. The polypeptide, or peptide, is then recovered and purified as necessary. Recovery and purification can be achieved using any of those procedures known in the art, for example by absorption onto the elution from an anion exchange resin. This method of producing a polypeptide, or peptide, of the invention constitutes a further aspect of the present invention.

Host cells transformed with the vectors of the invention also form a further aspect of the present invention.

Methods for chemical synthesis of nucleic acids are well known and can be performed, for example, on commercial automated oligonucleotide synthesisers.

The term "stringent conditions" is functionally defined with regard to the hybridisation of a nucleic acid probe to a target nucleic acid (for example, to a particular nucleic acid sequence of interest) by the hybridisation procedure discussed in Sambrook et al. (1989) at 9.52-9.55 and 9.56-9.58.

Regarding the amplification of a target nucleic acid sequence (for example,. by PCR) using a particular amplification primer pair, stringent conditions are conditions that permit the primer pair to hybridise only to the target nucleic acid sequence to which a primer having the corresponding wild type sequence (or its complement) would bind.

Nucleic acid hybridisation is affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary

strands, and the number of nucleotide base mismatches between the hybridising nucleic acids, as will be readily appreciated by those skilled in the art.

When referring to a probe or primer, the term "specific for (a target sequence)" indicates that the probe or primer hybridises under stringent conditions only to the target sequence in a given sample comprising the target sequence.

The term "protein (or polypeptide)" refers to a protein encoded by the nucleic acid molecule of the invention including fragments, mutations and homologs having the same biological activity (for example, insecticidal activity). The polypeptide of the invention can be isolated from a natural source, produced by the expression of a recombinant nucleic acid molecule or be chemically synthesised.

Peptides having substantial sequence identity to the above-mentioned peptides can also be employed in preferred embodiments. Here, "substantial sequence identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80% sequence identity, preferably at least 90% sequence identity, more preferably at least 95% sequence identity or more. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to effect the properties of a protein. Examples include glutamine for asparagine, or glutamic acid for aspartic acid.

BRIEF DESCRIPTION OF DRAWINGS

The invention will be further defined by reference to the specification and the following examples and figures herein.

Figure 1 shows restriction maps of clones used to isolate the pathogenic region and maps of the two pathogenic variants pMH32 and pMH41, in accordance

with a preferred embodiment of the present invention; and

Figure 2 shows deletion derivatives used in the study, restriction maps of the mutated constructs and recombinants, the phenotype of each mutation, the schematic diagram of the sequenced region, and a nucleotide sequence in accordance with a preferred embodiment of the present invention; and

Figure 3 shows hydrophobicity plots of SepC and its closest homologue TccC, in accordance with a preferred embodiment of the present invention; and

Figure 4 shows the comparison of protein sequences of the SepA and *P. luminescens* toxins, TcdA, TcaB and TccB Putative RGD motif is boxed, plus the site of proteolytic cleavage is illustrated, in accordance with a preferred embodiment of the present invention; and

Figure 5 shows the comparison of protein sequences of the SepC and *P. luminescens* toxin TccC, in accordance with a preferred embodiment of the present invention; and

Figure 6 shows the plasmid pADAP, in accordance with a preferred embodiment of the present invention.

BEST MODES FOR CARRYING OUT THE INVENTION

The invention will be further defined by reference to the specification and the following examples and figures herein in the ensuing description by way of example only where:

Figure 1 shows restriction maps of clones used to isolate the pathogenic region and maps of the two pathogenic variants pMH32 and pMH41, where:

(A) Is the pADAP *Hind*III clone pGLA-20 showing locations of the pGLA-20 mutations –

10, -13, and 35, which when recombined back into pADAP and bioassayed against grass grub, result in either a pathogenic phenotype, shown by full flag, or a healthy but non-feeding phenotype indicated by half filled flag. Map of pBG35 showing relative position of pGLA-20-35 mutation and the location of the 2.2kb *Eco*Ri used as a probe to screen the pADAP *Bam*HI library; and

(B) Illustrated restriction enzyme maps of the pathogenic clones pMH32 and pMH41, area of deletion is indicated by Δ.

pBR322 vector DNA;

pLAFR3 vector DNA.

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI: H, *Hind*III; and X, *Xba*I.

Figure 2 shows:

(A) Which are Mini-Tn*10* pACYC184 based deletion derivatives used in the study.

is the pACYC184 vector,

Δ indicates deletion + pathogenic,

- loss of pathogenicity; and

(B) Illustrates restriction maps of the mutated constructs pBM32 and the pADK recombinants; and

(C) Where the phenotype of each mutant is indicated by flags.

Blocked flags indicates mutations that did not affect the disease process.

Open flags indicate mutations that abolish disease symptoms.

Half-filled flags denote mutations that abolish visual disease symptoms but are unable to feed.

* indicates pADK mutations obtained by Grkovic et al. (1995).

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; and X, *Xbal.*

(D) Is a schematic diagram of the sequenced region, where:

Denotes sequenced region.

Arrows indicate ORFs and their direction

region homologous to spvB ... location of repeat.

(E) Is a nucleotide sequence of the 5 times 12bp repeat and the palindrome.

Restriction enzymes are abbreviated as follows: B, *Bam*HI, Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; and X, *Xbal.*

In Figure 3 hydrophobicity plots of SepC and its closest homologue TccC are shown. The scale is disproportional to size and has a scanning window of 17 amino-acid residues.

Figure 4 shows the comparison of protein sequences of the SepA and *P. luminescens* toxins, TcdA, TcaB and TccB. Putative RGD motif is boxed. The site of proteolytic cleavage is reported by Bowen et al. (1998) (Residue 1933 of TcdA) is indicated by an arrow.

Figure 5 shows the comparison of protein sequences of the SepC and *P. luminescens* toxin TccC; and Figure 6 shows the plasmid pADAP.

PROTOCOL

Bacterial isolates and methods of culture

Table 1 lists bacterial isolates and plasmids used in the present invention. Bacteria were grown in LB broth or on LB agar (Sambrook et al. 1989), at 37° for *Escherichia coli* and 30°C for *S. entomophila*. Antibiotic concentrations used (μg/ml) for *Serratia* were kanamycin 100, chloramphenicol 90, tetracycline 30 and for *E. coli* strains were kanamycin 50, chloramphenicol 30, tetracycline 15, and ampicillin 100.

DNA isolation and manipulations

pADAP DNA was isolated from a 50ml overnight culture of bacteria using QIAGEN® plasmid maxi kit (Qiagen, Hilden, Germany), as per the manufacturer's instructions. Standard DNA techniques were carried out as described by Sambrook et al. (1989). Radioactive probes were made using the Amersham Megaprime DNA labeling system (Amersham, Buckinghamshire, UK). Southern and colony hybridisations were performed as outlined in Sambrook et al. (1989). The plasmid pADAP is shown in Figure 6.

pADAP *Bam*HI library was constructed using a Sigma 'Gigapack®IIIXL packaging extract, as specified by the manufacturer (Stratagene, California, USA).

Introduction of plasmid DNA into *E. coli* and *S. entomophilia*

pLAFR3 based derivatives were introduced into *S. entomophilia* by tripartite matings on solid media as described previously (Finnegan & Sheratt, 1982) using the pRK2013 helper plasmid (Figorski & Helanski, 1979). pACYC184 and pBR322 based plasmids were electroporated into *E. coli* and *S. entomophilia* strains, using a Biorad Gene Pulser (2μF, 2.5KV, and 200 abns) (Dower et al. 1988).

Mutagenesis

Transposon insertions were generated in recombinant plasmids using the mini-*Tn10* derivative 103 (kanamycin resistant) as described by Kleckner et al. (1991). Insertions were recombined into pADAP by transforming A1MO2 (refer to Table 1) with the described construct. After growth in non-selective media, bacteria were screened for resistance to kanamycin and loss of the pLAFR3 tetracycline resistance marker.

Bioassay against *Costelytra zealandica* larvae

Infection of *C. zealandica* larvae was determined by a standard bioassay where the healthy larvae, collected from the field, were individually fed squares of carrot which had been rolled in colonies of bacteria grown overnight on solid media (resulting in approximately 10^5 cells/carrot square). Twelve, second or third instar larvae were used for each treatment. Inoculated larvae were maintained at 15°C, in ice-cube trays. Larvae were left feeding on treated carrot for 3-4 days, then transferred to fresh trays and provided with untreated carrot for 10-14 days. The occurrence of gut clearance and loss of feeding was recorded every 3-4 days. Strains were considered disease-causing if greater than 70% of larvae showed disease symptoms by day 14. Known pathogenic and non pathogenic controls were included in all bioassays. Typically cessation of feeding occurs within 2-3 days while clearance of the larvae gut may take 4-6 days.

Recovery of bacteria from larvae

To isolate bacteria from inoculated grubs, larvae were surface sterilised by submerging in 70% methanol for 30 seconds. The larvae were then shaken in sterile DH_20, removed and individually macerated in a 1.5ml microcentrifuge tube. The macerate was serial diluted and plated on LB media containing antibiotics selective for the host *S. entomophilia* strain. To assess the stability of the bioassayed plasmid, colonies were patched onto a plate

containing antibiotics either selective for the recombinant plasmid or the *S. entomophilia* strain. Identity of plasmids in the recovered strain was checked by restriction enzyme profile.

Nucleotide Sequencing

A 9-kb *Bam*HI –*Eco*RI fragment derived from the pBM32-8 mutation (Fig 2b) and the 8kb *Hind*III fragment of pBM32 were separately cloned into the appropriate site of the deletion factory plasmid pDELTA1. Deletions were generated using the Delection factory™ system (GIBCO BRL, MD, USA), as outlined in the manufacturers instructions. To identify the precise location of mini-*Tn10* mutations, the peripheral mini-*Tn10* *Bam*HI sites were used in conjunction with the *Bam*HI sites of the pathogenic region to subclone the mini-*Tn10* flanking regions into either pACYC184 or pUC19. Sequences were generated using the mini-*Tn10* specific primer 5'ATGACAAGATGTGTATCCACC3' (Kleckner et al. 1991).

Plasmids for sequencing were prepared by Wizard® (Promega, Madison, USA) or Quantum Prep® (Bio-Rad, California, USA) miniprep kits. Sequences were determined on both strands, by using combinations of subcloned fragments, custom primers and deletion products derived from the deletion factory system (Gibco BRL, Madison, USA). The DNA was sequenced using either ^{33}P dCTP and the Thermosequenase cycle sequencing kit (Amersham, Buckinghamshire, UK), or by automated sequencing using an Applied Biosystem 373A or 377 autosequencer. Sequence data were assembled using SEQMAN (DNASTAR Inc., Madison, USA). ORF's were analysed by Gene Jockey. Databases at the National Center for Biotechnology Information were searched by using BLASTN and BLASTX via the www.ncbi.nlm.gov/BLAST. Searches for DNA palindromes, repeats and inverted repeats were undertaken using DNAMAN (Lynnon Biosoft, Quebec, Canada). Protein motifs were searched using Blocks *(http://www.blocks.fhcrc.org/)*, ExPASy *(http://www.expasy.ch/)*, and Gene Quiz *(http://columba.ebi.ac.uk:8765/gqsrv/submit)*.

The sequences determined in this study have been deposited in Gene Bank under Accession Number AF1335182.

RESULTS

Cloning the disease encoding region from pADAP

Previously, Grkovic et al. (1995) have shown taht the pADK-13 mutation can be complemented with the pADAP 11 kb *Hind*III fragment (pGLA-20). However, the pADK-10 mutation was unable to be complemented with pGLA-20. In an attempt to isolate the region that may complement the pADK-10 mutation the previously described pGLA-20 derived, pADK-35 null mutation (Grkovic et al. 1995) was used as a selective marker (Fig 1), to select the *Bgl*II fragment encompassing both the pADK-10 and pADK-35 mutations. pADK-35 DNA was isolated and digested with the restriction enzyme *Bgl*II. The resultant digest was ligated into the *Bam*HI site of bBR322 to form the construct pBG35 (containing 12.8kb *Bgl*II – mini-*Tn10* fragment). pBG35 was placed separately in *trans* with pADK-10 and pGLA-20, and the resultant strains bioassayed against grass grub larvae. Results showed that pBG35 was able to complement the pADK-10 mutant, but was unable to induce any symptoms of amber disease when placed in *trans* with pGLA-20, indicating that there must be another region on pADAP needed to induce amber disease.

Restriction enzyme data of pGLA-20 and pBG35 suggested that the entire pathogenic region may reside within one of the large *Bam*HI fragments of pADAP. A cosmid *Bam*HI library of pADAP was made and screened using the 2.2kb *Eco*RI fragment derived from pBG35 (Fig 1) as the probe. Several probe positive clones were isolated; all shared similar restriction enzyme profiles. However, one (designated pMH32) was found to be smaller, measuring only 23kb in size compared with the 33kb of the other clones (eg. pMH41; Fig 1b). The difference between pMH32 and pMH41 was found to be a 10kb deletion at the left most end of pMH32 encompassing the one *Hind*III site (Fig 1). *E. coli* strains

containing pMH32 or pMH41 were bioassays against grass grub larvae and found to induce the full symptoms of amber disease (that is - gut clearance and antifeeding activity). However, about ten days after infection a proportion of grass grubs fed the *E. coli* strains were found to recover from a diseased to a healthy phenotype.

The plasmids pMH32 and pMH41 were subsequently introduced into a *S. entomophilia* strain cured of pADAP (5.6RC) and the strains bioassayed against grass grub larvae. The strains gave the same disease progression as wild type and no larvae recovered, suggesting that the region cloned in pMH32 contained all the pathogenic determinants of pADAP.

Effect of copy number and mini-*Tn10* insertions in pBM32 on disease-causing ability

To facilitate mutagenesis and assess the effect of copy number on the disease process, the 23kb *Bam*HI fragment from pMH32 was cloned into the medium copy plasmid pBR322 to give pBM32. A bioassay comparing the ability of pMH32 and pBM32 to induce amber disease against grass grub was undertaken. Results showed that there were no visual differences in the progression of amber disease between pBM32 and pMH32. The construct pBM32 was mutated with the mini-*Tn10* transposon derivative 103, and insertions mapped (Fig 2b). Bioassays of *E. coli* strains containing plasmids of the resultant mutants, showed that the disease determinants were confined within a central 16.9kb region (nucleotides 1955-18937 of SEQ ID NO: 1).

All strains were non-pathogenic or fully pathogenic, and no partial disease phenotypes such as antifeeding, or gut clearance were noted.

To confirm that no sequences at either end of the cloned fragment influenced the disease process, several deletion plasmids were made (Fig 2a). The large fragments resulting from cleavage of the pBM32 -4, -8, -10, -20, -23, -24 and -35 plasmids with *Bam*HI were cloned into the analogues site of pACYC184. The resultant plasmids were transformed into the

non-pathogenic *S. entomophilia* strain 5.6RKm and assessed for pathogenicity. This analysis confirmed that the central 16.9kb region (Fig 2a) was sufficient to induce the disease.

Effect of mini-*Tn10* insertions in pADAP on disease-causing ability

Grkovic et al. (1995) recombined by marker exchange the pGLA-20 based mutations - 10 and -13 into pADAP (Fig 2a). When bioassayed, *S. entomophilia* strains containing either of these mutant plasmids caused a partial condition including cessation of feeding but not gut clearance or amber colouration. This was in contrast to the complete abolition of disease observed in pADAP-cured *S. entomophilia* strains containing mutant pBM32 plasmids with similar insertions.

To determine the disease phenotype of the pBM32-based insertions in a pADAP background, the pBM32 based insertions were transferred into pADAP. pBM32 -1, -2, -4, -5, -6, -8, -9, -10, -21, -24, -30, -31 and -35 DNA fragments containing the inserted transposon and flanking DNA were cloned as independent fragments into pLAFR3 and the inserts recombined back into pADAP by marker exchange (Fig 2c). The resultant recombinant *S. entomophilia* strains were checked by Southern analysis to confirm that recombination had occurred as expected and no pLAFR3 vector sequences were present (data not shown). Mutations that did not affect the disease process in pBM32 also had no effect when recombined back into pADAP. However, strains with the pADAP mutants that totally abolished the disease process when in the pBM32 clone caused non-feeding but not gut clearance of the grubs (Fig 2b, c). Hence, none of the pADAP recombinant strains completely abolished the disease process. This suggests that, while the 16.9kb fragment contains all genes required for pathogenicity, other genes contributing to the antifeeding effect are present on some other part of pADAP.

Assessment of plasmid stability during the course of the bioassay showed that greater than

90% of the recombinant *Serratia* strains contained the clone of interest.

Nucleotide Sequence Analysis of the pathogenic region

The large *Bam*HI fragment (18937 bp) derived from the pBM32-8 was sequenced on both strands using a combination of constructed detections, plasmid subclones and custom made primers. A total continuous sequence of 18937 bp has been deposited in Gene Bank (Accession Number AF135182). Structural analysis of the DNA sequence using DNAMAN showed that there was a 12-bp sequence repealed five times between positions 683 and 743. The repeat is flanked by an upstream 13 base pair palindrome (669-682-bp), and a degenerate 34-bp downstream palindrome (765-799-bp)(Fig 2d,e).

Translation of the nucleotide sequence revealed nine significant open reading frames (ORF's). These together with their putative ribosomal binding sites and their base composition are listed in Table 2. Eight of the ORF's were oriented in the same direction and the other two in the opposite direction (Fig 2d). Sequence similarity searches showed that the deduced products of seven of these ORF's shared similarity with known proteins (Table 3). Products of three of the ORF's showed similarity to different protein components of insecticidal toxins of *Photorhabadus luminescents* (Bowen et al. 1998).

These ORF's have been designated *sep. (sepA, sepB and sepC)* for *Serratia entomophilia* pathogenicity.

Similarities of deduced amino-acid sequences to proteins in current database

Results of database searches for homologues proteins are listed in Table 4.

With reference to Fig 2d and Table 4, the following protein similarities were identified:

The protein product of *sepA,* had high similarity to the *P. luminescents* insecticidal toxin complex protein TcbA, TcdA, TcaB and TccB. These proteins shared three significant

regions of predicted amino-acid similarity, at the amino-terminal region (SepA amino-acid residues (121-178), a central region (SepA amino-acid residues 960-1083) and, with greatest similarity, at the carboxyl terminus (SepA amino-acid residues 1630-2376) Fig. 4). However, there was little amino acid conservation around the putative proteolytic cleavage site of TcaB, TcbA and TcdA identified by Bowen et al. (1998). SepA also contained a region (residues 1057-1345) with weak similarity to the *Clostridium bifermentans* mosquitocidal toxin cbm71 (Barloy et al., 1996).

SepB and the *P. luminescens* insecticidal toxin complex protein TcaC shared similarity throughout their length, and both SepA and TcaC showed high amino-terminal similarity to the *Salmonella* virulence protein spvB (Gullig et al. 1992) (Fig. 5). The similarity of SepB and TcaC to SprB diminishes after SpvB amino acid residue 356.

SepC showed strong similarity to the amino-terminal of the insecticidal toxin complex protein TccC, up to amino-acid residue 663 of SepC. A number of putative bacterial cell wall proteins also have high similarity to SepC, including the wall associated protein precursor *B. subtilis* (WAPA) and members of the *E. coli Rhs* (recombinant hot spot) elements. Strong similarity of SepC was also observed with hypothetical wall-associated proteins from *Coxiella burnetti* and *Bacillus subtilis* (Table 4).

The translated sequences of ORF1 and ORF2 showed no similarity to sequences in the current databases. ORF3 shared significant similarity to the morphogenesis protein of the *Bacillus subtilis* bacteriophage B103, a member of bacteriophage muramidase-type lysis proteins (Pecenkova et al. 1996). However, relative to size, the gp19 protein of *S. typhimurium* phage ES18 (146 amino-acid residues) or the nucD/regB phage lysozymes of *S. marcescens* (179 amino-acid residues) are more similar. ORF4 showed similarity to *E. coli* bacteriophage N15gp 55 protein, a protein of unknown function (Zimmer et al. 1998).

Located in the same orientation as the sep genes and 134bp downstream of the *SepC*

termination codon is a 204 base pair region assigned ORF5, which has high similarity to a *S. typhimurium* revolvase/invertase protein. However ORF5 is disrupted by two stop codons at amino-acid residues 19 and 64, making it unlikely that an active resolvase/invertase protein, is encoded by this region. A 256-bp region of encompassed by ORF5 (17498-17754) showed high similarity (77% identity) to the region (AF020806; 1629-1885 bp) encoding *S. typhimurium* DNA invertase gene (Valdivia et al. 1997) suggesting a similar ancestral origin.

Downstream of ORF5 and oriented in the opposite direction from 18935-18163 was a 870 base pair region of DNA designated ORF6 whose product showed high amino-acid similarity over two different reading frames to the insertion element *IS*91 of *E. coli* (Mendiola et al. 1992). The translated sequence is interrupted at amino-acid residue 149 of the *IS*91 element and later resumed on a second reading frame, before its similarity switched back to the original reading frame. Swtiching of ORF's is a common feature of members of the IS3 family where the transposase is encoded by this overlapping ORF's (Prere et al. 1990). However, the switch back to the initial strand is atypical. ORF6 may therefore be a dysfunctional relic of an ancestral *IS* element. It is unknown whether ORF6 contains a ribosomal binding site as its predicted location would lie outside the sequenced region. There was no DNA similarity to the *IS*91 element.

Analysis for protein motifs showed that a tripeptide cell-binding motif Asp-Gly-Arg (RGD), implicated in the binding of various adhesion proteins produced by parasites and viruses to eukaryotic cells (Leininger et al. 1991), is present in SepA and the *P. luminescens* TcdA, and TcaB proteins (Fig. 4). The RGD motif is present in cell surface adhesions produced by the human pathogen *Bordetella pertussis*, namely the filamentous heamagglutinin (220 kDa) (Relman et al. 1989), and the outer membrane protein pertactin (69 kDa) (Leininger et al. 1991). These motifs have been implicated in enhancing the binding of *B. pertussis* to eukaryotic cells. Because the RGD motif found in SepA falls in a

region of high similarity between SepA and its *P. luminescens* counterparts, it may play a role in meditating the attachment of the protein and/or the bacteria to the insect cell wall.

The hydropathicity profile of each of the Sep proteins was examined using the Kyte and Doolittle algorithm (Kyte and Doolittle, 1982) and compared to the relevant *P. luminescens* homologues. None of the Sep proteins contained a positively charged amino terminus followed by a hydrophobic region, characteristic of a signal sequence (Gierasch, 1989). The profiles of SepA, TcbA and TcdA were very similar (data not shown) and each exhibited a steep hydrophilic peak at the carboxyl terminus (residues 2055-2061 of SepA), specifically the protein sequence RRRRE (Fig. 4). Although both SepB and TcaC shared similarity to the *Salmonella* virulence protein SpvB, the amino-terminil of SepB and TcaC were hydrophilic as opposed to the hydrophobic nature of SpvB. The profile of SepC and its *Photorhabadus* counterpart TccC differed in that SepC had a slightly hydrophilic amino-terminus, whereas TccC lacked a hydrophilic amino-terminus and had a significantly hydrophobic carboxyl terminus from amino-acid residue 717 onwards (Fig. 3).

Analysis to detect repetitive motifs characteristic of the RTX family of toxins (Welch, 1991) using DOTPLOT showed only *P. luminescens* TccC contained a plot characteristic of a repeat motif present at the carboxy terminal (data not shown).

Analysis of DNA composition (%GC) and similarity

Comparisons of the GC content (Table 3) showed that the *SepA* and *SepB* genes were more GC-rich than their *P. luminescens* counterparts, while *SepC* and *tcaC* had similar GC content. The high GC content of *SepC* may be attributed to the close relationship of these protein products to the *rhs* family of wall-associated proteins which have a GC-rich core of 62% (Wang et al. 1998). Comparisons of the GC content of the *Sep* genes with that of the *S. entomophilia* genome shows that they are rather similar, suggesting that the *sep* genes were not recently acquired by *S. entomophilia*.

Identification of mini-*Tn10* location by sequence analysis

Analysis of the insertion points of the previously isolated mini-*Tn10* insertions (Fig. 2) within the putative ORF's (Table 4) revealed that ORF3 and ORF4 were interrupted by the -9, -23, -24 (ORF3) and -35 (ORF4) mutations. These insertions had no effect on the pathogenicity process, suggesting that ORF3 and ORF4 do not play a significant role in pathogenicity. However, the pADAP-35 mutation was at the 3' end of ORF4, resulting in the truncation of the final 11 amino-acid residues of ORF4 (Fig. 4), which may not have affected protein function. Further mutagenesis of ORF4 is therefore required to confirm that it has no role in pathogenicity. The mutations that caused loss of pathogenicity all resided within *SepA*, *SepB* or *SepC*. No mutation mapped to ORF1, ORF2 or ORF5.

Complementation analysis of the sep proteins

Following sequence data each of the *Sep* ORF's were excised as closely as possible with restriction enzymes, placed into pLAFR3 and placed in *trans* with the appropriate pADAP mutation. Complementation of *SepA* was undertaken through the use of the 8.5 kb *Hind*III clone (pMH45) which encompasses both ORF1 and *SepA*. *SepB* was excised as a 5.4 kb *Stu*I fragment and *SepC* was excised as a 4.6 kb fragment using one of the peripheral; *Bam*HI sites from the pBH32-13 mutation and the *Stu*I site of pBM32 (Fig. 2b).

Complementation analysis showed that pLAFR3 based *SepB* and *SepC* are able to complement their mutated pADK- counterparts. Grkovic et al. (1995) had already previously shown that *SepC* could complement itself. However, this was achieved through using the entire 11 kb *Hind*III, pGLA-20 fragment.

Whether *SepA* is able to complement itself has yet to be fully established. It was found that ~98% of the pMH45 construct was lost during the course of the bioassay. This latter result was sporadic and occasionally a repeated experiment would show the presence of diseased

grubs. Analysis of the macerates of these grubs showed that pMH45 was present indicating that pMH45 can possible complement *SepA*. However before further complementation analysis of *SepA* can be undertaken, measures to ensure the complementation plasmids stability are needed.

DISCUSSION

The large conjugative plasmid, pADAP, of *S. entomophilia* encodes the genes responsible for cessation of feeding and gut clearance, characteristics of amber disease in the New Zealand grass grub *C. zealandica*. This plasmid is present in all *S. entomophilia* and *S. proteamaculans* strains capable of causing amber disease (Glare et al. 1993) and had been implicated in disease processes (Grkovic et al. 1995). The applicant has defined a 16.9 kb region of kADAP that is sufficient to confer pathogenicity towards *C. zealandica* on pADAP-cured strains of *S. entomophilia* and on strains of *E. coli*. Hence, the region confers all the essential pathogenicity genes of *S. entomophilia* responsible for amber disease. Nucleotide sequence and mutagenesis analysis of the region revealed three genes, *SepA*, *SepB* and *SepC*, that together are sufficient for pathogenicity. Mutations in any of the three genes completely abolished the disease process and partial disease states were not detected, suggesting that the three genes may interact to exert an effect.

The 23-kb region cloned into pBR322 to make pBM32 conferred pathogenicity in pADAP-cured *S. entomophilia* strains with all symptoms of amber disease being observed. Insertion mutants in pBM32 that abolished pathogenicity were transferred to pADAP. The resultant strains showed a partial disease phenotype, including anti-feeding but not gut clearance, suggesting that an additional anti-feeding gene may be present elsewhere on pADAP. The occurrence of two different anti-feeding genes on pADAP also supports data of Grkovic et al. (1995) who found that suppression of feeding was stronger in the wild-type pADK-6 strain, compared to the partial disease state (pADK-10, pADK-13) of

inducing anti-feeding but no gut clearance. A putative anti-feeding gene, *amb2*, has already been isolated from the genomic DNA of *S. entomophilia* (Nunez-Valdez and Mahanty, 1996). Recent data indicate that the *amb2* locus resides at an as yet to be identified location on pADAP that is remote from the region identified herein (Hurst, unpublished data).

Sequence analysis and comparison of the products of the *sep* genes showed that they share significant similarity to the proteins TcbA (TcdA, TcaB, TccB), TcaC and TccC that comprise the toxin complexes of *P. luminescens*. Like the *P. luminescens* genes that *sep* genes of *Serratia* share a similar organisational pattern of three genes ordered in succession in the same orientation, and opposed by a terminil gene transcribed in the opposite direction. However, the order of sep genes differ, are slightly smaller in size, and comprise constituents of each of the *P. luminescens* loci tca (tcaB=sepA, tcaC=sepB), *luminescens* toxin gene tcd (Ensign et al. 1997) is also similar to SepA. The similarity shared between the *sep* and *tc* gene products suggests that they are members of a new family of insecticidal toxins. The lack of DNA similarity as opposed to protein similarity between *sep* and *P. luminescens* tc genes together with the differnce in GC content of the *sepA* and *sepB* genes compared to the tc genes, suggests that these genes were present in the common enterobacterial ancestor of *P. luminescens* and *S. entomophilia* and were not acquired by a more recent horizontal transfer event.

The *Photorhabadus* toxins were isolated as a composite of proteins which are hypothesised to interact synergistically to form a toxin complex. The toxins are also able to exert an anti-feeding effect (Bowen et al. 1998; Bowen and Ensign, 1998). This is consistent with the results we obtained with the *sep* mutants. pADAP-cured *S. entomophilia* strains containing the pathogenicity clone pBM32 exert an anti-feeding effect on the grass grub and individual mutations within any of the *sep* genes have an identical phenotype, completely abolishing pathogenicity. The *Photorhabadus* toxins have a wide host range, affecting Lepidoptera, Coleoptera and Dictyoptera and undergo post translational

proteolytic processing (Bowen et al. 1998). No similarities of *sep* proteins were found to the *Photorhabadus* toxin component TccA, and only the amino-terminus of TcaA shared similarity to *SepA*. This and the difference in the hydrophobicity profiles of *SepC* and TccC, may account for specificity of the *sep* proteins towards *C. zealandica*. However the *sep* proteins have yet to be purified and it is unknown whether the *sep* genes are expressed when *S. entomophilia* is ingested by other insects. Therefore the possibility that these newly-described toxins may exhibit a broader host range cannot be ruled out.

The *Photorhabdus* toxin TcbA shares weak similarity to the *Clostridium difficile A* and *B* toxins (Bowen, 1998), but no such similarities were found to *SepA*. *C. difficile A* and *B* toxins belong to the RTX (repeats in toxin) family of toxins which are noted for the presence of several carboxyl terminal repeats (von Eichel-Streiber et al. 1992). A search of the *sep* proteins and their *P. luminescens* homologues for protein repeats showed that only the *P. luminescens* TcaC protein contained a repeat-type signature. The TcaC carboxy-terminal repeat bears little resemblance in size or number of repeats found in RTX toxins (von Eichel-Streiber et al. 1992). *SepA* does not show weak similarity to the mosquitocidal toxin Cbm71 of *C. bifermentans* (Barloy et al. 1996). However when this region is compared with the relevant *Photorhabdus* homologues, it is a region with little similarity.

SepB has strong similarities to both *P. luminescens* TccC and the *Salmonella* virulence gene product SpvB (Gulig et al. 1992). SpvB is believed to enhance the survival of virulent *Salmonella* in macrophages (Libby et al. 1997). It has been suggested that TcaC may act by attacking insect haemocytes (Bowen et al. 1998). However, haemocytes reside within the insect haemocoel and *S. entomophilia* does not invade the haemocoel until late in the infection process (Jackson et al. 1993), suggesting that *SepB* may act in some other way. The similarity of *SepB* and TcaC is high to SpvB but diminishes ten amino-acid residues upstream of the proline-rich region found in SpvB that is postulated to divide the protein into separate domains (Roudier et al. 1992). This may indicate a vital role for the amino-

terminus of both *SepB* and SpvB in interacting with an evolutionarily-conserved eukaryotic protein.

The *SepC* protein shows high similarity to a family of cell wall-associated bacterial proteins such as the *B. subtilis* wall-associated protein (WAPA) and members of the *E. coli* rhs element family. The function of the Rhs proteins has yet to be established, but they are believed to be cell surface ligand-binding proteins (Hill et al. 1994). The Rhs proteins and the *B. subtilis* was-associated protein contain a characteristic repetitive peptide motif, but no such motif was observed in *SepC*. A feature of rhs elements is the presence of a downstream IS element (Wang et al. 1998). A degenerate IS91-type transposase element (ORF6) is present downstream of *SepC*. The IS91 element has been found associated with plasmids or chromosomal genes involved in α-haemolysin synthesis, and has been postulated to play a pivotal role in the spread of the α-haemolysin genes by means of the IS91-mediated recombinational activity (Zabala et al. 1984). It seems possible an IS element adjacent to *SepC* may have been involved in the acquisition of the *sep* genes by *S. entomophilia.*

Blackburn et al. (1998) undertook histological examinations of the lepidopteran *Manduca sexta* after treatment with the *P. luminescens* Tca toxin complex introduced by feeding or haemcoelic injection. They found blebbing of the midgut epithelium into the lumen, resulting in lysis and formation of cavities. Similar histological studies have been undertaken at various stages throughout the infection cycle of *S. entomophilia* in *C. zealandica,* and reveal a visible deterioration in the number of fat cells to almost minimal levels, and an emptying of the larval gut. However no blebbing of the midgut epithelium was observed (Jackson et al. 1993).

The *S. entomophilia* pathogenicity region endows pathogenicity on members of the Enterobacteraceae such as *Klebsiella spp., Enterobacter agglomerans, E. coli,* and *Serratia*

species (Glare et al. 1996). From this we can infer that the *Sep* proteins are the major virulence determinants, that the promoters of the *sep* genes are expressed constitutively or under the control of conserved regulatory genes, or a negative regulatory gene present in the pathogenicity region, and that export of the toxin proteins is carried out by a conserved chromosomally encoded system, or is an intrinsic property of the *sep* proteins. The *Sep* proteins have no obvious amino terminal signal sequences, a facet shared with E-Group colicins. The release of cloacin DF13 is mediated through a small lipoprotein designated BRP, for bacteriocin-release protein. Low level expression of BRP in conjunction with phospholipase A leads to the release of cloacin DF13, along with bacterial periplasmic proteins. However if expressed in high amounts, BRP causes cell death by cell lysis (vad der Wal, 1998). The close proximity and similar orientation pattern of ORF3 to the *sep* genes indicate that ORF3 may have an as yet to be determined important functional role. Protein similarity searches show that it has high similarity to the bacteriophage lysozyme family. In relation to amino-acid size, ORF3 closely resembles the LZBP22 lysozyme of the *Salmonella* P2 bacteriophage, a protein essential for the lysis of the bacterial cell wall (Rennell and Poteete, 1985). It is possible that ORF3 may facilitate the release of the *sep* proteins by lysing the bacterial cell wall. A low level expression of ORF3 might, as in the case of BRP, allow the passage of the *sep* proteins across the cell wall without causing cell death. The reason that the pBM32-9 and -24 mutations were unable to abolish the disease process could be due to a masking of ORF3 function by natural cell lysis of the bacteria.

A region of repetitive DNA was identified between nucleotides 683 to 743, centered within a 1.2-kb AT rich stretch of DNA that contains no potential ORF's. The repeat motif is flanked by an upstream 13-bp palindrome and a degenerate downstream 33-bp palindrome. Repeats have been found to be common sites for recombination (Allgood et al. 1988), or to facilitate the binding of proteins. A 66-bp DNA sequence termed the *rsk* element for reduced serum killing, of the *S. typhimurium* 95-kb virulence plasmid, comprises of a series

of direct 10-bp repeats with a 21 nucleotide periodicity. The *rsk* element is believed to titrate out a *trans*-acting factor, enhancing the expression of the *Salmonella* serum resistance gene (Vandenbosch et al. 1989). It is not known whether these repeats and/or flanking palindromes have a role in the pathogenicity process. The deletion derivative pAC24, which encompasses this region, was still pathogenic towards the grass grub. However, this deletion could also unknowingly remove the complete regulatory circuit of the pathogenicity region, leading to constitutive expression.

THE ARABINOSE EXPRESSION SYSTEM

Methodology

Using the polymerase chain reaction (PCR) the initiation codon ATG of the three *sep* genes (*sepA*, *sepB* and *sepC*) were individually placed into the unique *Nde*I site (restriction enzyme site CATGG) of the HIS-tag arabinose expression vector pAV2-10 (obtained from Chuck Shoemaker –AgResearch). Because large proteins i.e. greater than 50 kda are limited in their ability to bind to HIS tag affinity columns the carboxyl terminus of each of the Sep proteins did not need to be in frame with the HIS-tag site. Instead wild type DNA (non PCRd) containing a downstream chloramphenicol resistance gene was ligated into the appropriate restriction enzyme site (*sepA Sun*I; *sepB Hin*dIII; *sepC Bst*XI) of the pAV2-10-*sep* derived vectors:-

-the use of the chloramphenicol resistant marker provided by the vector pACYC184 enhances the stability to each of the expression constructs i.e. -the antibiotic ampicilin to which the pAV2-10 is resistant too is cleaved in the media to an inactive form leading to possible plasmid free segregants arising. Conversely the antibiotic chloramphenicol is not cleaved heightening the level of plasmid stability under conditions of arabinose induction.

To validate the legitimacy of the fused genes to the arabinose expression vector, PCR generated products and the ligation junctions were verified by DNA sequencing.

Concurrent to this the *sepB* and *sepC* genes were placed as derived from pADAP downstream of *sepA*. Also *sepA*, *sepB* and *sepC* were placed as in pADAP downstream of orf3. This simulated wildtype conditions (i.e. the arrangement of the *sep* genes on pADAP) and hopefully get the production of the *sep* genes and the complex driven off the one upstream promoter. A method which Western analysis has shown to be successful –with moderate levels of *sepA*, *sepB* and *sepC* being detected.

The arabinose expression system is one of the tightest systems known with almost complete abolition of gene product under arabiniose free conditions Guzman *et al.* (1995), this abolition can be enhanced by providing glucose to the medium. In contrast providing arabinose at the concentration of 0.2% will switch the arabinose promoter on express any genes under its control e.g. *sepA* etc. Typically an overnight culture of the *E. coli* strain was set up the next day an 100 µl of the culture was suspended in fresh media supplemented with chloramphenicol (30 µg/ml) the culture was grown until an OD of 400 at which time arabinose was added to the culture to a final concentration of 0.2% and the culture left shaking at 30 °C for 18 hours.

To date Western analysis has shown that each of the proteins is expressed and expressed to its correct predicted size:

SepA 262.7 kdal

SepB 156.6 kdal

SepC 107 kdal

SepC is expressed at high levels with minor levels of proteolytic cleavage. However both SepA and SepB though expressed are cleaved in high amounts by endogenous *E. coli* proteases. Alternative strains of *E. coli* are going to be assessed for loss of proteolytic activity against SepA and SepB

It has also been shown that placing all three of the *sep* genes under the control of a single arabinose promoter will result in the production of basil levels of the SepA, SepB, SepC toxin complex.

Each of the following Coleopteran species were mouth injected with 3-5 µl of an overnight suspension of induced bacteria (*E. coli* strain DHB101) containing either SepA, SepB and SepC or orf3, SepA, SepB and SepC.

Each larvae was then given a $3mm^3$ piece of carrot coated with a 50% solution (dH_20) of arabinose. Observations were noted each day and the larvae refed with a $3mm^3$ piece of carrot coated with a 50% solution (dH_20) of arabinose

Red headed cock chaffer

Tasmanian grass grub

Odontara

Grass grub (positive control)

Under these conditions it has been found that the arabinose expressed toxin complex SepA, SepB and SepC is active against grass grub but not any of the other species of scarabs tested (see above). It is therefore thought unlikely that the toxin complex will have activity to other insect orders.

SUMMARY

The bacteria *Serratia entomophilia* and *S. proteamaculans* cause amber disease in the grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae), an important pasture pest in New Zealand. Larval disease symptoms include amber colouration, clearance of the gut and rapid cessation of feeding, before eventual death. The region containing pathogenic determinants of the disease has been cloned, and further defined by mutagenesis and deletion analysis to a 16.9 kb region. Sequence analysis of the minimal pathogenic encoding region showed significant protein homology, but little sequence homology to a group of newly described toxins from a member of the Enterobacteriaceae, *Photorhabadus luminescens.* This pathogenicity-encoding region from *S. entomophilia* plasmid pADAP is the subject of the invention. The proteins encoded by the genes (*sepA, sepB, sepC)* within the 16.9 kb region can be used for insect control whether as an inundative pesticide, within baits or expressed in other organisms such as plants or microbes.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope thereof as defined in the appended claims.

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Table 1 Bacterial strains, plasmids and bacteriophage used in the study

Bacteria	Description	Reference
Escherichia coli		
DH5α	F⁻ ϕ80d *lacZ*ρM15 ρ(*lacZYA-argF*)U169 *recA1 endA1* supE44	Hanahan (1983)
DH10B	F⁻ *mcrA* ρ(*mrr-hsd*RMS-*mcr*BC)ϕ80d *lacZ*ρM15 ρ*lacX74 endA1 recA1 deo*Rρ(*ara, leu*) 7697 *ara*D139 *gal*U *gal*K *nup*G *rps*L λ⁻.	Lorow and Jessee, (1990)
DF1	γδ transposase(*tnpA*)	Gibco BRL
MC1061	*sup*ᵒ *hsdR mcrB araD*139 ρ(*araA BC-leu*)7679 ρ*lacX74 galU galK rpsL thi*	Casadaban and Cohen, (1980)
MC4100	*araD139* ρ(*lacZYA-argF*)U169 *rpsL150* St^R *relA1 flbB5301 deoC1 ptsF25 rbsR*	Silhavy *et al.* (1984)
XL1-BlueMRA	ρ(*mcrA*)183 ρ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 reA1 gyrA96 relA1*	Stratagene
Serratia entomophila		
A1MO2	Ap^R, pADAP, pathogenic.	Grimont *et al.* (1988)
5.6	heat cured pADAP minus derivative of A1MO2	Glare *et al.* (1993)
5.6RC	Cm^R *recA*⁻ pADAP minus strain	Grkovic *et al.* (1996)
5.6RK	Kn^R *recA*⁻ pADAP minus strain	this study
Plasmids		
pACYC184	Cm^R Tc^R	Chang and Cohen, (1978)
pADAP	Amber disease associated plasmid	Glare *et al.* 1993)
pBR322	Ap^R, Tc^R	Bolivar *et al.* (1977)
pBM32	23-kb *Bam*HI fragment from pMH32 cloned in pBR322	this study
pBM32-1-40	pBM32 containing mini-*Tn10* insertions	this study
pDELTA1	Ap^R, Sm^R, Kn^R, sucrose^R	Gibco BRL
pLAFR3	Tc^R pRK290 with λ*cos*, *lacZ*α and multi-cloning site from pUC8.	Staskawicz *et al.* (1987)
pRK2013	IncP, Kn^R Tra RK2 *repRK2 repE1*	Ditta *et al.* (1980)
pGLA20	10.6-kb *Hin*dIII pADAP fragment cloned in pLAFR3	Corbett (unpublished)
pACρ4	19-kb *Bam*HI fragment from pBM32-4 cloned in pACYC184	this study
pACρ8	17-kb *Bam*HI fragment from pBM32-8 cloned in pACYC184	this study
pACρ10	19.5-kb *Bam*HI fragment from pBM32-10 cloned in pACYC184	this study
pACρ20	20-kb *Bam*HI fragment from pBM32-20 cloned in pACYC184	this study
pACρ23	21-kb *Bam*HI fragment from pBM32-23 cloned in pACYC184	this study
pACρ24	21.2-kb *Bam*HI fragment from pBM32-24 cloned in pACYC184	this study
pADK-10	pADAP::mini-Tn*10* insertion in 10.6-kb *Hin*dIII fragment, Kn^R non-pathogenic	Grkovic *et al.* (1995)
pADK-13	pADAP::mini-Tn*10* insertion in 10.6-kb *Hin*dIII fragment, Kn^R non-pathogenic	Grkovic *et al.*(1995)
pADK-35	pADAP::mini-Tn*10* insertion in 10.6-kb *Hin*dIII	Grkovic *et al.* (1995)

	fragment, Kn^R, pathogenic	
pMH32	23-kb *Bam*HI frgament of pADAP cloned into pLAFR3	this study
pMH41	33-kb *Bam*HI fragment of pADAP cloned into pLAFR3	this study
pBM32	23-kb *Bam*HI fragment of pMH32 cloned into pBR322	this study
pUC19	Ap^R, *lacZ*α, multi-cloning site	Yannish-Perron, *et al.* (1985)
Bacteriophage		
λNK1316	mini-Tn*10* derivative 103 donor λb522 c1857 Pam80 nin5	Kleckner *et al.* (1991)

Table 2 Position of genes and features of the predicted gene products encoded by *sep* genes

ORF	Putative ribosome-binding site[a]	Longest potential coding region		*sep* %GC (*P. luminscens* homologue, %GC)
		Start at nucleotide	Stop at nt (ORF size bp)	
sepA	ATGGGACCATCAACGTAATGAA TGAGG	2413	9547 (7131)	54 (*tcbA*, 43; *tcdA*, 44)
sepB	CGAGGAGACTGAGCATGCAA	9598	13885 (4287)	58 (*tcaC*, 51)
sepC	ACAGGAGATCACATGAGC	14545	17467 (2922)	55 (*tccC*, 54)
ORF1	CATAGAGACTGTCGCTATGTTA	1287	1587 (300)	39
ORF2	TTGGAGAATAACCGCCATGTT	1590	1863 (273)	39
ORF3	GGGGGAGAAAAATGAAG	1860	2294 (435)	51
ORF4	TGACTGGGAAGGAGGGGGGGAC GGTGATGAGT	13908	14483 (576)	60
ORF5	TAACGAGACTTTTTAGCAAAAT GGCACTTT	1761-1755, 1755-1773		?
ORF6	GAGCATGGC-Mini-Tn*10*-8[b]	18934-18064		?

[a] Putative ribosome-binding sites are underlined, and potential start codons are in boldface; nt, nucleotides; ? degenerate or incomplete ORF. [b] ORF transcribed in opposing direction.

Table 3. Comparisons of GC content between the *Sep* and *P. luminescen* genes

Sep (%GC)	*P. luminescen* toxin (%GC)
sepA (54%)	*tcbA* (43%) *tcdA* (44%)
sepB (58%)	*tcaC* (51%)
sepC (55%)	*tccC* (54%)

Table 4. Similarities f pr ducts of putative ORF's to protein sequences in the database detected using BlastP

ORF (a.a size)	Protein homo-logue (a.a size)	Degree of similarity %identity/%similarity (over) a.a residue – a.a residue	Function of the homologous protein	Organism	Blast score Reference[■]
SepA (2373)	TcbA (2504)	34/50 (1675) 41-1628* 57/72 (751) 1630-2374*	insecticidal toxin complex protein	*Photorhabdus luminescens*	0.0 AF047457
	TcdA (2405)	40/55 (2458)*	insecticidal toxin complex protein	*P. luminescens*	0.0 Ensign *et al.*, (1997)
	TcaB (1189)	38/54 (764) 1625-2374* 29/50 (281) 936-1198*	insecticidal toxin complex protein	*P. luminescens*	e^{-137} AF046867
	TccB (1565)	36/51 (859) 1575-2373* 31/51 (289) 930-1204*	insecticidal toxin complex protein	*P. luminescens*	e^{-136} AF047028
	TcaA (1095)	36/56 (90) 94-183* 18/39 (530) 435-928*	insecticidal toxin complex protein	*P. luminescens*	$1e^{-8}$ AF046867
	TccA (965)	27/45 (186) 115-280*	insecticidal toxin complex protein	*P. luminescens*	$5e^{-6}$ AF047028
	Cbm71 (613)	24/41 (199) 1057-1250*	Mosquitocidal toxin Cbm71	*Clostridium bifermentans*	g2127309
SepB (1428)	TcaC (1485)	49/63 (1276)1-1263* 64/78 (152) 1270-1421*	insecticidal toxin complex protein	*P. luminescens*	0.0 AF046867
	SpvB (591)	40/52 (357) 9-365*	*Salmonella* virulence protein	*Salmonella typhimurium*	$4e^{-62}$ S22664
SepC (938)	TccC (1043)	53/66 (836) 3-782*	insecticidal toxin complex protein	*P. luminescens*	0.0 AF047028
	SC2H4.02 (2183)	23/34 (639) 68-677*	Hypothetical wall associated protein	*Streptomyces coelicolor*	$2e^{-12}$ AL031514.1
	WapA (2334)	22/34 (430) 255-677* 20/36 (613) 48-625*	Wall associated protein Precursor	*B. subtilis*	$2e^{-5}$ S32920
	Y15898 (334)	21/34 (542) 181-684*	hypothetical wall associated protein	*Coxiella burnetii*	$9e^{-5}$ Y15898
	Rhs core (1420)	21/35 (463) 237-677* 21/36 (285) 35-300*	Rhs core protein	*E. coli*	$3e^{-4}$ AF044501
ORF3 (144)	BB103G (263)	45/62 (142) 1-139*	morphogenesis protein of bacteriophage B103	*Bacillus subtilis*	$3e^{-27}$ CAA67646
	LZBP22 (146)	46/61 (139) 1-143	Phage P22, lysozyme (E 3.2.1.17)	*Salmonella*	$1e^{-24}$ gi 138699
ORF4 (191)	Gp55 (181)	28/42 (188) 1-184*	bacteriophage N15 protein	*E. coli*	$1e^{-6}$ AF064539
ORF5 (236)	SprA	75/79(68) 1-68 ♦	Resolvase/invertase homologue	*S. typhimurium*	$7e^{-19}$ AF029069 AF020806
ORF6 (310)	IS*91*	39/56 (94) 130-197♦ -1* 39/58 (94) 224-318♦ -2* 30/48 (76) 319-395♦ -1*	IS*91* transposase	*E. coli*	$4e^{-28}$ S23782

Percent identities and similarities were calculated in relation to the deduced gene products of the sequenced ORF. *indicates position of amino-acid similarity in relation to sequence generated in this study. ♦ indicates position of amino-acid similarity in relation to data base protein sequence. * reading frame. ■ similarities were considered potentially significant if the BlastP score exceeded e^{-5}.

Table 5 **Positions of mini-Tn*10* insertions**

Mini-Tn*10* insertion #	ORF	Position downstream of initiation codon (bp)
9/23	ORF3	120
24	ORF3	345
4	*sepA*	747
27	*sepA*	1037
40	*sepA*	1097
6	*sepA*	1727
38	*sepA*	2887
2	*sepA*	3197
5	*sepA*	3737
3	*sepA*	3697
19	*sepA*	3697
30	*sepA*	4467
37	*sepA*	4467
31	*sepA*	4627
12	*sepB*	182
22	*sepB*	172
11	*sepB*	362
10	*sepB*	2162
35	ORF4	557
13	*sepC*	2525
8		18937
ORF4/-35 junction GGG CGC *TGA TGA ATC*		

THE CLAIMS DEFINING THE INVENTION ARE:

1. A purified and isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 1 that encodes at least one of:

   (i) an insecticidal protein complex, or

   (ii) a functional fragment of said complex, or

   (iii) a neutral mutation of said complex, or

   (iv) a homolog of said complex,

   each of which have at least 75% nucleic acid homology to SEQ ID NO: 1 and are capable of hybridising with said nucleic acid molecule under stringent hybridisation conditions.

2. A purified and isolated nucleic acid molecule as claimed in Claim 1 comprising the nucleotide sequence 1995-18937 of SEQ ID NO: 1.

3. A purified and isolated nucleic acid molecule as claimed in Claim 1 comprising one or more of the nucleotide sequences 2411-9547, 9589-13883 or 14546-17467 of SEQ ID NO: 1.

4. A purified and isolated nucleic acid molecule as claimed in Claim 3 comprising all of nucleotide sequences 2411-9547, 9598-13884 and 14546-17467 of SEQ ID NO: 1.

5. A purified and isolated nucleic acid molecule as claimed in Claim 1 comprising a sequence of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

6. A purified and isolated nucleic acid molecule as claimed in Claim 2 comprising nucleotides 1955-18937 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

7. A purified and isolated nucleic acid molecule as claimed in Claim 3 comprising a sequence of SEQ ID NO: 1,or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

8. A purified and isolated nucleic acid molecule as claimed in any one of claims 4 through 6 wherein the said nucleotide sequence includes the nucleotide sequence which codes for at least one of the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins.

9. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein nucleic acid molecule may comprise DNA, cDNA or RNA.

10. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein the nucleic acid molecules said fragment, neutral mutation or homolog thereof capable of hybridising to said nucleic acid molecule, hybridise to the nucleotide sequence of SEQ ID NO: 1, or nucleotides 1955-18937, 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1 if there is at least 75% or greater identity between the sequences.

11. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein the nucleic acid molecule may be isolated from *Serratia entomophila* or *Serratia proteamaculans* strains of bacteria.

12. A recombinant expression vector(s) containing the nucleic acid molecule as claimed in Claim 1 and host transformed with the vector expressing a polypeptide.

13. A recombinant expression vector(s) as claimed in claim 11 wherein the vector is selectable from any suitable natural or artificial plasmid/vector.

14. A recombinant expression vector(s) as claimed in claim 13 wherein said suitable natural or

artificial plasmid/vector, including, pUC 19 (Yannish-Perron et al. 1995), pProEX HT (GibcoBRL, Gaithersburg, MD, USA), pBR322 (Bolivar et al. 1977), pACYC184 (Chang et al. 1978), pLAFR3 (Staskowicz et al. 1987).

15. A polypeptide resulting from the transformation or transfection of a host cell with a recombinant expression vector as claimed in any one of Claims 12 through 14.

16. A method of producing a polypeptide of claim 15 comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with said vector as defined above to express the encoded polypeptide or peptide; and

(b) recovering the expressed polypeptide or peptide.

17. The use of a ligand that binds to a polypeptide of claim 15 to isolate and/or identify the polypeptide of claim 15.

18. An antibody or antibody binding fragment that binds to a polypeptide of claim 15.

19. Probes and primers comprising a fragment of the nucleic acid molecule as claimed in Claim 1 wherein said fragment is hybridisable under stringent conditions to a native insecticidal gene sequence.

20. Probes and primers comprising a fragment of the nucleic acid molecule as claimed in claim 19 wherein said probes and primers enable the structure and function of the gene to be determined and homologs of the gene to be obtained from bacteria other than *Serratia* sp.

21. A polypeptide as claimed in Claim 15 wherein the polypeptide has insecticidal activity encoded by the nucleic acid molecule of claim 1, or a functional fragment, neutral mutation or homolog thereof.

22. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide

comprises the amino acid sequence of SEQ ID NO: 1 or a functional fragment, neutral mutation or homolog thereof.

23. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide comprises amino acids 32-5118 of SEQ ID NO: 1.

24. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide comprises at least one amino acid sequence of SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5 or SEQ ID NO: 6.

25. A polypeptide having insecticidal activity as claimed in claim 24 wherein the polypeptide preferably comprises amino acid sequence SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6.

26. A polypeptide having insecticidal activity as claimed in claim 24 wherein the polypeptide preferably comprises all of SEQ ID NOs: 2-6.

27. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide is obtained by expression of a DNA sequence coding therefore in a host cell or organism.

28. A polypeptide having insecticidal activity as claimed in claim 27 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 1 linked to at least one further amino acid sequence encoding an insecticidal protein.

29. A polypeptide having insecticidal activity as claimed in claim 28 wherein the at least one further amino acid sequence includes the amino acid sequence which codes for *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescents* toxins.

30. A polypeptide having insecticidal activity as claimed in claim 28 wherein the polypeptides comprise at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity to SEQ ID NO: 1.

31. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide is produced by expression of a vector comprising the nucleic acid of SEQ ID No:1 or a functional fragment, neutral mutation or homolog thereof, in a suitable host cell.

32. An insecticidal composition comprising at least the polypeptide as claimed in claim 21 and an agriculturally acceptable carrier.

33. An insecticidal composition as claimed in claim 32 wherein more than one polypeptide is included in the composition.

34. An insecticidal composition as claimed in claim 32 or 33 wherein the composition comprises additional pesticides, including compounds known to possess herbicidal, fungicidal, insecticidal or nematicidal activity.

35. An insecticidal composition as claimed in claim 34 wherein the composition comprises other known insecticidally active agents, including *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescents* toxins.

36. A method of combating pests, said method comprising applying to a locus, host and/or the pest, an effective amount of the polypeptide as claimed in Claim 21 that has functional insecticidal activity against said pest.

37. A method of inducing amber disease or like condition in insects comprising delivery to an insect an effective amount of the polypeptide as claimed in Claim 21 that has functional insecticidal activity against said insect.

38. A method of inducing amber disease or like condition in insects as claimed in claim 37 comprising delivery to an insect an effective amount of the polypeptide wherein the insect is selected from the order comprising Coleoptera.

39. A method of inducing amber disease or like condition in insects as claimed in Claim 38

comprising delivery to an insect an effective amount of the polypeptide wherein the insect includes *Costelytra zealandica* (Coleoptera: Scarabaeidae).

40. A method of delivering the insecticidal polypeptide to induce amber disease or like condition in insects including delivery of the insecticidal polypeptide as claimed in Claim 39 to the insect by any one of presenting the insecticidal polypeptide orally as a solid bait matrix, as a sprayable insecticide sprayed onto a substrate upon which the insect feeds, applied directly to the soil subsurface or as a drench or is expressed in an transgenic plant, bacterium, virus or fungus upon which the insect feeds.

41. A transgenic plant, bacterium virus or fungus, incorporating in its genome, a nucleic acid molecule as claimed in Claim 1 for providing the plant, bacterium virus or fungus with an ability to express an effective amount of an insecticidal polypeptide.

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ccgttagcta taaatatgat atttaaatct gtatttttat ataaaaccag tttatgatgc 1080

tggattggtc attaaagtcg ttatatgtga tcgttatctg tcattgattg gtgtttaatc 1140

ttttattctt ccagtgaggt ttcaggggga atgtattggg taatcatact catgtcattt 1200

gttgctttga tgttaaatta acgtgttcat tcattatgtt ctactgttgt ttctattgtc 1260

cggaacgacc atagagactg tcgctatgtt aataggaata tttgactggt tatatgcgcc 1320

aagggttatc gctgcactct ctggggcgat ggtattcatc attacgcaag ataacttcat 1380

tggtgtcaga cgggtgttat tgtttttttgt gtctttttta ctcggtttga cattttcaga 1440

gacaacagct tccgttatca acttctatat cccgaatgat atacatatag gaaatgacct 1500

tggtgccttt gttaccagcg ccgtgacggt gaagcttttt gttatcatta tgagcaagat 1560

agagagaaaa tatcttggag aataaccgcc atgttccaaa tcatacttct taatgttaat 1620

gccgtgattt gcttggctat tgccgtcaga ttattcctgt ggcgtatcaa tcataaaatg 1680

aaaaacattg tcgtctcttt tattgctttt ctcattatta cggcgtgcgg cgctgtctcc 1740

atcaggacga tgacggggga gtattactat gcggattggt ccgagacgat cattaacctt 1800

tcgcttttcc tgtctgttta tatacgcaat ggcgaaatcc ttcggtgggg ggagaaaaa 1859

atg aag ata agt tcc cga ggt atc gca tta atc aaa gag ttc gaa ggt 1907
Met Lys Ile Ser Ser Arg Gly Ile Ala Leu Ile Lys Glu Phe Glu Gly
  1               5                  10                  15

ctg cgc tta cac gct tat cgc tgc gcc gct gac gtc tgg act gtc ggt 1955
Leu Arg Leu His Ala Tyr Arg Cys Ala Ala Asp Val Trp Thr Val Gly
             20                  25                  30

tat ggc cac acg gca ggg gtt aca aag ggt gac atc atc acg gtc gat 2003
Tyr Gly His Thr Ala Gly Val Thr Lys Gly Asp Ile Ile Thr Val Asp
         35                  40                  45

gaa gcc cag acg atg ctg aca aac gat att acc gta ttt gaa cgg gcg 2051
Glu Ala Gln Thr Met Leu Thr Asn Asp Ile Thr Val Phe Glu Arg Ala
     50                  55                  60

gtc agt cag gcc gtc gcg gtt cct ctg aat cag tcg caa tac gat gcc 2099
Val Ser Gln Ala Val Ala Val Pro Leu Asn Gln Ser Gln Tyr Asp Ala
 65                  70                  75                  80

ctg gtt tct ttg gtt ttt aat att ggc cag ggg aat ttt aaa cgc tct 2147
Leu Val Ser Leu Val Phe Asn Ile Gly Gln Gly Asn Phe Lys Arg Ser
                 85                  90                  95

acc ttg ttg aaa aaa ctc aac aaa cag gac tat gtc ggc gcc ggg aac 2195
Thr Leu Leu Lys Lys Leu Asn Lys Gln Asp Tyr Val Gly Ala Gly Asn
             100                 105                 110

gag ttt tta cgc tgg acc cgg gcc aat ggg aag gtc ctt ccc gga ctg 2243
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Glu Phe Leu Arg Trp Thr Arg Ala Asn Gly Lys Val Leu Pro Gly Leu
        115                 120                 125

att cgc cga cgc gaa gct gaa cgg gtg ttg ttt gag aaa ctg ggt gca   2291
Ile Arg Arg Arg Glu Ala Glu Arg Val Leu Phe Glu Lys Leu Gly Ala
    130                 135                 140

taa ccctttgcga cgtacccaca agatgaagat aacaccgcgt actgagcggt       2344

145

ggcgcaacaa tgaataaatg actgtgtacg gcctgtcctt cacaacggat gggaccatca 2404

acgtaa tga atg agg caa gac att atg tat aat att gat gat att ctg    2452
          Met Arg Gln Asp Ile Met Tyr Asn Ile Asp Asp Ile Leu
                    150                 155

gag aaa gtg aat gct cca cga gca cgc ctg tca gaa gaa aac gat aca   2500
Glu Lys Val Asn Ala Pro Arg Ala Arg Leu Ser Glu Glu Asn Asp Thr
160                 165                 170                 175

gcg gtg acg ctg acg gat tta ttc tcg cgt tcg ttt ccc gag gtc aaa   2548
Ala Val Thr Leu Thr Asp Leu Phe Ser Arg Ser Phe Pro Glu Val Lys
                180                 185                 190

aaa atc act ggc gac agc ctg tca tgg gga gag gtc tgc tat ctg tac   2596
Lys Ile Thr Gly Asp Ser Leu Ser Trp Gly Glu Val Cys Tyr Leu Tyr
            195                 200                 205

agt cag gcg cag cac gaa cag aaa gaa aac cgg ctc acc gaa tcc cgt   2644
Ser Gln Ala Gln His Glu Gln Lys Glu Asn Arg Leu Thr Glu Ser Arg
        210                 215                 220

att ctg gcc cgg gcg aat ccc cta ctg gtg aat gcc gtt cgc ctg gga   2692
Ile Leu Ala Arg Ala Asn Pro Leu Leu Val Asn Ala Val Arg Leu Gly
    225                 230                 235

ata cgg cag gca gcc ggc agt cgc agc tat gat gac tgg ttt ggc tcc   2740
Ile Arg Gln Ala Ala Gly Ser Arg Ser Tyr Asp Asp Trp Phe Gly Ser
240                 245                 250                 255

cgc gca gac cgt ttc gcc cgc ccc ggc tcg gtg gcc tcc atg ttc tca   2788
Arg Ala Asp Arg Phe Ala Arg Pro Gly Ser Val Ala Ser Met Phe Ser
                260                 265                 270

ccg gcg gcg tat ctg acc gag ctg tac cgt gag gcg aag gac ctg cat   2836
Pro Ala Ala Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asp Leu His
            275                 280                 285

ccg gac acc tcg ctg ttc cgg ctg gac atc cgg cgt ccc gac ctg gcg   2884
Pro Asp Thr Ser Leu Phe Arg Leu Asp Ile Arg Arg Pro Asp Leu Ala
        290                 295                 300

gcg ctg gcc ctt agc cag aat aat atg gac gac gag ctc tcc acc ctg   2932
Ala Leu Ala Leu Ser Gln Asn Asn Met Asp Asp Glu Leu Ser Thr Leu
    305                 310                 315

agc ctg tcc aat gag cta ctg tat cgc ggt atc ggg gca gcg gaa ggg   2980
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Ser Leu Ser Asn Glu Leu Leu Tyr Arg Gly Ile Gly Ala Ala Glu Gly
320                 325                 330                 335

ctt gac gac gac agc gtc agg gag ctg ctc gcc ggg tat cgc ctg acc   3028
Leu Asp Asp Asp Ser Val Arg Glu Leu Leu Ala Gly Tyr Arg Leu Thr
                340                 345                 350

ggc ctg acc ccc tat cac tgg gcg tac gag gcg gcc cgc caa gcc att   3076
Gly Leu Thr Pro Tyr His Trp Ala Tyr Glu Ala Ala Arg Gln Ala Ile
            355                 360                 365

ctg gtg cag gac ccg acg ctg atg ggg ttc agc cgt aat ccg gat gtg   3124
Leu Val Gln Asp Pro Thr Leu Met Gly Phe Ser Arg Asn Pro Asp Val
        370                 375                 380

gcg cag ctt atg gac cct gcc tcc atg ctg gcc att gaa gcc gat att   3172
Ala Gln Leu Met Asp Pro Ala Ser Met Leu Ala Ile Glu Ala Asp Ile
    385                 390                 395

tca ccg gag ctg tat cag ata ctg gcc gaa gaa att acg aca gac agt   3220
Ser Pro Glu Leu Tyr Gln Ile Leu Ala Glu Glu Ile Thr Thr Asp Ser
400                 405                 410                 415

tac gaa gca ctc tgg agt aag aat ttt ggt gat atg cct ccc tcc tca   3268
Tyr Glu Ala Leu Trp Ser Lys Asn Phe Gly Asp Met Pro Pro Ser Ser
                420                 425                 430

ctg tta tct tat gat gca ctt gca aca ttt tat gat ctt gat tac gat   3316
Leu Leu Ser Tyr Asp Ala Leu Ala Thr Phe Tyr Asp Leu Asp Tyr Asp
            435                 440                 445

gag cta act tcg tta ttg tca tta agg ctg gac ttt tca aat cca aac   3364
Glu Leu Thr Ser Leu Leu Ser Leu Arg Leu Asp Phe Ser Asn Pro Asn
        450                 455                 460

aat gaa tac tac att aat agt caa tta agt gtc gta act ctg aat gaa   3412
Asn Glu Tyr Tyr Ile Asn Ser Gln Leu Ser Val Val Thr Leu Asn Glu
    465                 470                 475

agc act ggt tta ata act ata cat cat tat tta aga acg cta ggc gga   3460
Ser Thr Gly Leu Ile Thr Ile His His Tyr Leu Arg Thr Leu Gly Gly
480                 485                 490                 495

gac tca cag cag att aac cct gag ctt ata cct tat ggg gat gga aca   3508
Asp Ser Gln Gln Ile Asn Pro Glu Leu Ile Pro Tyr Gly Asp Gly Thr
                500                 505                 510

tat ctt tat aat ttc agc gtg gtg tca acg ata tca gag gat agt ttc   3556
Tyr Leu Tyr Asn Phe Ser Val Val Ser Thr Ile Ser Glu Asp Ser Phe
            515                 520                 525

aaa cta ggg tcg tta ggt tct aac agt agc aat ctt tac tct ggg gat   3604
Lys Leu Gly Ser Leu Gly Ser Asn Ser Ser Asn Leu Tyr Ser Gly Asp
        530                 535                 540

tat cag ctt caa aaa ggg gtt cgc tat agc att cct gtt gaa ata gat   3652
Tyr Gln Leu Gln Lys Gly Val Arg Tyr Ser Ile Pro Val Glu Ile Asp
    545                 550                 555
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gaa gga aag tta aat gat ggg atc aca ata gga ttg agt agg aaa ggg   3700
Glu Gly Lys Leu Asn Asp Gly Ile Thr Ile Gly Leu Ser Arg Lys Gly
560                 565                 570                 575

ggg gga tat tac tca aca gta aac ttc act ctg att gaa tat gat cct   3748
Gly Gly Tyr Tyr Ser Thr Val Asn Phe Thr Leu Ile Glu Tyr Asp Pro
                580                 585                 590

gcg ata ttc att ctt aaa tta aat aaa gtt atc cgc cta tac aag gcc   3796
Ala Ile Phe Ile Leu Lys Leu Asn Lys Val Ile Arg Leu Tyr Lys Ala
            595                 600                 605

acg ggc atg acc acg gcg gaa ata tat caa atc acc aat att ctt aat   3844
Thr Gly Met Thr Thr Ala Glu Ile Tyr Gln Ile Thr Asn Ile Leu Asn
        610                 615                 620

aac ggt ctc acc att gac cat gcg gtc ctg agt aaa atc ttc ctg gtc   3892
Asn Gly Leu Thr Ile Asp His Ala Val Leu Ser Lys Ile Phe Leu Val
    625                 630                 635

cgt tac ctg atg cgt cac tat cag ctt gat gtg gcc cgg tca ctg ata   3940
Arg Tyr Leu Met Arg His Tyr Gln Leu Asp Val Ala Arg Ser Leu Ile
640                 645                 650                 655

ttg tgc aac gga acc atc agt gac cag gcg ttc agc ggc gaa acc ggc   3988
Leu Cys Asn Gly Thr Ile Ser Asp Gln Ala Phe Ser Gly Glu Thr Gly
                660                 665                 670

ctg ttc acc acg ctg ttc aac acc cca ccg ctg aac ggc cag ctg ttt   4036
Leu Phe Thr Thr Leu Phe Asn Thr Pro Pro Leu Asn Gly Gln Leu Phe
            675                 680                 685

tct gca gat gat acc ccc ctc gac tta cgc tct gaa gca ccg gag gat   4084
Ser Ala Asp Asp Thr Pro Leu Asp Leu Arg Ser Glu Ala Pro Glu Asp
        690                 695                 700

gct ttc cgt ctc agc gta ctg aaa cgc gca ttt aac atc agc gcc tcg   4132
Ala Phe Arg Leu Ser Val Leu Lys Arg Ala Phe Asn Ile Ser Ala Ser
    705                 710                 715

ggg ctt tcc acg ctc tgg cag ttg gcc agc ggt gac agc agc gct ggg   4180
Gly Leu Ser Thr Leu Trp Gln Leu Ala Ser Gly Asp Ser Ser Ala Gly
720                 725                 730                 735

ttt agc tgc tct gct gac aat atc gcc gca ctc tac cga gtg aaa ctc   4228
Phe Ser Cys Ser Ala Asp Asn Ile Ala Ala Leu Tyr Arg Val Lys Leu
                740                 745                 750

ctg gct gac atc cac gac cta tcc gct ggt gag ctg tca atg ttg ctg   4276
Leu Ala Asp Ile His Asp Leu Ser Ala Gly Glu Leu Ser Met Leu Leu
            755                 760                 765

tcc gtc tcc cct ttc agc ggg gtg gcc gcc ggc tcg ctg tcc gat aat   4324
Ser Val Ser Pro Phe Ser Gly Val Ala Ala Gly Ser Leu Ser Asp Asn
        770                 775                 780

gag ctg acg cag ttt ctg tac cag acc acc acc tgg ctc acg gag cag   4372
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Glu Leu Thr Gln Phe Leu Tyr Gln Thr Thr Thr Trp Leu Thr Glu Gln
    785                 790                 795

ggc tgg acg gtc agc gat gtg ttc ctg atg ctg acg acg cag tac ggt   4420
Gly Trp Thr Val Ser Asp Val Phe Leu Met Leu Thr Thr Gln Tyr Gly
800                 805                 810                 815

acc ctg ctg acc ccc gac att gag aac ctg ctc gct tcc ctg cgc aac   4468
Thr Leu Leu Thr Pro Asp Ile Glu Asn Leu Leu Ala Ser Leu Arg Asn
                820                 825                 830

gga ctg tcg ggc cgt gag ctg ttc ccg gaa acg ctc ccc ggc gat ggc   4516
Gly Leu Ser Gly Arg Glu Leu Phe Pro Glu Thr Leu Pro Gly Asp Gly
            835                 840                 845

gct ccc ttt att gcc gcc gcc atg cag ctg gac gcc acg gat acg gcg   4564
Ala Pro Phe Ile Ala Ala Ala Met Gln Leu Asp Ala Thr Asp Thr Ala
        850                 855                 860

aag gcg atg ctg act tgg gcg gac cag ttg aag cca gag ggg ctg acg   4612
Lys Ala Met Leu Thr Trp Ala Asp Gln Leu Lys Pro Glu Gly Leu Thr
    865                 870                 875

ctg acg gaa ttt att ctt ttg gtg atg aat gcc gcc cca aat gac gag   4660
Leu Thr Glu Phe Ile Leu Leu Val Met Asn Ala Ala Pro Asn Asp Glu
880                 885                 890                 895

cag gcg ggc cag atg gca ggg ttc tgc caa gcc ctg tgg caa ctg gca   4708
Gln Ala Gly Gln Met Ala Gly Phe Cys Gln Ala Leu Trp Gln Leu Ala
                900                 905                 910

ctg atc atc cgc agc acc ggc ctc agc acg cgc gag ctg acg ctg ctg   4756
Leu Ile Ile Arg Ser Thr Gly Leu Ser Thr Arg Glu Leu Thr Leu Leu
        915                 920                 925

gtc agc cag ccg gga cgc ttc cgc aca gga tgg cac cat ctg ccc cat   4804
Val Ser Gln Pro Gly Arg Phe Arg Thr Gly Trp His His Leu Pro His
    930                 935                 940

gac ctg ccg gcg ctt cgc gac att acg cgt ttt cat gcc gtc gtt aac   4852
Asp Leu Pro Ala Leu Arg Asp Ile Thr Arg Phe His Ala Val Val Asn
945                 950                 955

cgc agc ggc agc cat gcc ggg gag gtc ctg acc gca ctt gag acc gga   4900
Arg Ser Gly Ser His Ala Gly Glu Val Leu Thr Ala Leu Glu Thr Gly
960                 965                 970                 975

gaa ctg tcg tca gcc ctg ctg gcc cgg gcc ctg tca cag aat gag cag   4948
Glu Leu Ser Ser Ala Leu Leu Ala Arg Ala Leu Ser Gln Asn Glu Gln
                980                 985                 990

gat gtg acc ggc gcc ttg gcg cag gtg agg ggg gcc ggt gaa cag gac   4996
Asp Val Thr Gly Ala Leu Ala Gln Val Arg Gly Ala Gly Glu Gln Asp
            995                1000                1005

aac agc gtg ttc acc tcc tgg gaa gag gtg gac cag gct gag cag tgg   5044
Asn Ser Val Phe Thr Ser Trp Glu Glu Val Asp Gln Ala Glu Gln Trp
        1010                1015                1020
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ctg gac atg agt gag acc ctg tcc att acg cca tcc ggt ctg gct agc   5092
Leu Asp Met Ser Glu Thr Leu Ser Ile Thr Pro Ser Gly Leu Ala Ser
   1025                1030                1035

ctg att gcc ctg aag tac atc aat gtg tcc gat gac agt gca ccg ttg   5140
Leu Ile Ala Leu Lys Tyr Ile Asn Val Ser Asp Asp Ser Ala Pro Leu
1040               1045                1050                1055

tac agc cag tgg cag gtg gta tcc ggt ctg ctg cag gcc ggg ctg aaa   5188
Tyr Ser Gln Trp Gln Val Val Ser Gly Leu Leu Gln Ala Gly Leu Lys
               1060                1065                1070

agc agc cag agc tcg gcg ctg cac gat tat ctg gag gag ggg acc agc   5236
Ser Ser Gln Ser Ser Ala Leu His Asp Tyr Leu Glu Glu Gly Thr Ser
          1075                1080                1085

agc gcc ctt tgt gcg tat tat ctg cgt aat ctg gca ccg aac atg gta   5284
Ser Ala Leu Cys Ala Tyr Tyr Leu Arg Asn Leu Ala Pro Asn Met Val
       1090                1095                1100

tcc ggg cgc gat gac ctc ttc ggg tat ctg ctg ctg gat aat cag gtg   5332
Ser Gly Arg Asp Asp Leu Phe Gly Tyr Leu Leu Leu Asp Asn Gln Val
   1105                1110                1115

tca gcc aag gta aaa acc acc cgc att gcg gag gcc atc gcc ggc ata   5380
Ser Ala Lys Val Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Gly Ile
1120               1125                1130                1135

cgg ctg tat atc aac cgg gcc ctt aac gga ata gaa ctc agc gcc atg   5428
Arg Leu Tyr Ile Asn Arg Ala Leu Asn Gly Ile Glu Leu Ser Ala Met
               1140                1145                1150

gca gag gtg agg ggg cgt cag ttt ttc act gac tgg gat acg ttc aac   5476
Ala Glu Val Arg Gly Arg Gln Phe Phe Thr Asp Trp Asp Thr Phe Asn
          1155                1160                1165

aaa cgt tac agc acc tgg gcg ggc gtc tca gag ctg gtt tac tat ccg   5524
Lys Arg Tyr Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro
       1170                1175                1180

gaa aac tac ctc gac ccg acg gtc cgt atc ggg cag acc ggc atg atg   5572
Glu Asn Tyr Leu Asp Pro Thr Val Arg Ile Gly Gln Thr Gly Met Met
   1185                1190                1195

gac acc ctg ctg cag tct gtc agc cag agc agt atc aac cgc gat acc   5620
Asp Thr Leu Leu Gln Ser Val Ser Gln Ser Ser Ile Asn Arg Asp Thr
1200               1205                1210                1215

gtg gag gat gcc ttt aaa acc tat ctg acc acg ttt gag cag att gcc   5668
Val Glu Asp Ala Phe Lys Thr Tyr Leu Thr Thr Phe Glu Gln Ile Ala
               1220                1225                1230

aat ctg aac act gtc agc gga tat cac gat aac gcc agc atg acg cag   5716
Asn Leu Asn Thr Val Ser Gly Tyr His Asp Asn Ala Ser Met Thr Gln
          1235                1240                1245

ggg act aca tgg tat gtg ggt cgc agc atc aca gat cag act aac tgg   5764
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Gly Thr Thr Trp Tyr Val Gly Arg Ser Ile Thr Asp Gln Thr Asn Trp
      1250                1255                1260

tac tgg cgc agc gcc aac cac agc aaa atc caa gac tca atg atg ccc   5812
Tyr Trp Arg Ser Ala Asn His Ser Lys Ile Gln Asp Ser Met Met Pro
   1265                1270                1275

gcg aat gcc tgg acc gga tgg aca aaa att aac tgc gga atg aat ccg   5860
Ala Asn Ala Trp Thr Gly Trp Thr Lys Ile Asn Cys Gly Met Asn Pro
1280                1285                1290                1295

tgg tca gat ctt gtg tgc tcg gtg ttt ttc aac agt cgc ctt tat gtc   5908
Trp Ser Asp Leu Val Cys Ser Val Phe Phe Asn Ser Arg Leu Tyr Val
               1300                1305                1310

gtc tgg gtc gaa gag aat cag tct gct gat acg gag gca gag agc acg   5956
Val Trp Val Glu Glu Asn Gln Ser Ala Asp Thr Glu Ala Glu Ser Thr
           1315                1320                1325

aca acc acg cag cag agc tac acg ctg aaa ctg tcg ttc cgg cgc tac   6004
Thr Thr Thr Gln Gln Ser Tyr Thr Leu Lys Leu Ser Phe Arg Arg Tyr
       1330                1335                1340

gac ggt aca tgg agt tcc ccg gtg tcg ttc gac att acc ggc aac atc   6052
Asp Gly Thr Trp Ser Ser Pro Val Ser Phe Asp Ile Thr Gly Asn Ile
   1345                1350                1355

gca ttt ccg gaa acg cag ggc atg cat gtg acc tgt aat ccc ctg act   6100
Ala Phe Pro Glu Thr Gln Gly Met His Val Thr Cys Asn Pro Leu Thr
1360                1365                1370                1375

gag cag ctc tat tgc gcg ttt tac tcc gtc acc agc aag ccg gac ttt   6148
Glu Gln Leu Tyr Cys Ala Phe Tyr Ser Val Thr Ser Lys Pro Asp Phe
               1380                1385                1390

gat aac gct cag ctg att tct gtg gat aat gat atg acg cta aat gtc   6196
Asp Asn Ala Gln Leu Ile Ser Val Asp Asn Asp Met Thr Leu Asn Val
           1395                1400                1405

atc tca gat ata ggg att ttt aag agc gtc agt cac gaa ttt aat acg   6244
Ile Ser Asp Ile Gly Ile Phe Lys Ser Val Ser His Glu Phe Asn Thr
       1410                1415                1420

agc act gag aaa ttt att aat aat gtt ttt tca gac cct tcc gct aat   6292
Ser Thr Glu Lys Phe Ile Asn Asn Val Phe Ser Asp Pro Ser Ala Asn
   1425                1430                1435

tat ttt gtc agt gca acg agt tta att gat gat gtt atc cac agc gat   6340
Tyr Phe Val Ser Ala Thr Ser Leu Ile Asp Asp Val Ile His Ser Asp
1440                1445                1450                1455

ttc tca ctc ctt aat tct aaa act aca agt act gtt ttt act aat gaa   6388
Phe Ser Leu Leu Asn Ser Lys Thr Thr Ser Thr Val Phe Thr Asn Glu
               1460                1465                1470

gat tcc tct ctt ttg acg cca gag ctt cat att aca gca aat gtt tcg   6436
Asp Ser Ser Leu Leu Thr Pro Glu Leu His Ile Thr Ala Asn Val Ser
           1475                1480                1485
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tgt ttt gtt agt act gct ggc atc gcc act caa tct acc ata gaa aaa   6484
Cys Phe Val Ser Thr Ala Gly Ile Ala Thr Gln Ser Thr Ile Glu Lys
       1490                1495                1500

ttc gtt cag gca ggg ata gaa ttt gag gaa att aat ttt tat gca ggc   6532
Phe Val Gln Ala Gly Ile Glu Phe Glu Glu Ile Asn Phe Tyr Ala Gly
   1505                1510                1515

cag gcc gcc ggc gga ttt gac gga ttt gtg gga gtg gat gtt tct aat   6580
Gln Ala Ala Gly Gly Phe Asp Gly Phe Val Gly Val Asp Val Ser Asn
1520                1525                1530                1535

tca aaa gta tac cag gtc gga aaa gaa gca gtt ggt gtc act gta aaa   6628
Ser Lys Val Tyr Gln Val Gly Lys Glu Ala Val Gly Val Thr Val Lys
               1540                1545                1550

tct tat tcc gtc act ggc gtt agt ggt tct gtt gag tta ttt att gat   6676
Ser Tyr Ser Val Thr Gly Val Ser Gly Ser Val Glu Leu Phe Ile Asp
          1555                1560                1565

tca tca aat aaa tac ttc agc gga att ttg tca gat aaa atg ata acc   6724
Ser Ser Asn Lys Tyr Phe Ser Gly Ile Leu Ser Asp Lys Met Ile Thr
       1570                1575                1580

gct tta att agc ggc agt aca tca aaa gtt aat tac gtg tcg tct att   6772
Ala Leu Ile Ser Gly Ser Thr Ser Lys Val Asn Tyr Val Ser Ser Ile
   1585                1590                1595

ggc tct caa gat ttt tgg agt gta aag tcg ctc atg ccg gca ctt cag   6820
Gly Ser Gln Asp Phe Trp Ser Val Lys Ser Leu Met Pro Ala Leu Gln
1600                1605                1610                1615

ata tat gaa tta atc gat gat atc ata ctg aca tcc ggc gta aat ggg   6868
Ile Tyr Glu Leu Ile Asp Asp Ile Ile Leu Thr Ser Gly Val Asn Gly
               1620                1625                1630

act gaa att aaa tcc tgg cct tcc gct gaa tgg tat aat gat aag ctg   6916
Thr Glu Ile Lys Ser Trp Pro Ser Ala Glu Trp Tyr Asn Asp Lys Leu
          1635                1640                1645

agt ctg caa tcc ggg aat aat ctt ttc aac acc aaa tcg ctg agt ttt   6964
Ser Leu Gln Ser Gly Asn Asn Leu Phe Asn Thr Lys Ser Leu Ser Phe
       1650                1655                1660

acc gtt aat acc agt gat att gtt gaa gat gag ttt gac gtg acg ttt   7012
Thr Val Asn Thr Ser Asp Ile Val Glu Asp Glu Phe Asp Val Thr Phe
   1665                1670                1675

acg ttc acc gct gtc gat cag aat aac gtc gtg ctg gcc gcc cgg acg   7060
Thr Phe Thr Ala Val Asp Gln Asn Asn Val Val Leu Ala Ala Arg Thr
1680                1685                1690                1695

gcc ata tta acc gtc att cga aac att aat aat gac act tcc gtt atc   7108
Ala Ile Leu Thr Val Ile Arg Asn Ile Asn Asn Asp Thr Ser Val Ile
               1700                1705                1710

gca tta cgt aaa aat acg cgt ggc gcg cag tat att cgt ttc act gcg   7156
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Ala Leu Arg Lys Asn Thr Arg Gly Ala Gln Tyr Ile Arg Phe Thr Ala
          1715                1720                1725

ggt aac gat gtg gcg ctt att cgc ctc aac acc ctc ttt gcc cgc caa   7204
Gly Asn Asp Val Ala Leu Ile Arg Leu Asn Thr Leu Phe Ala Arg Gln
       1730                1735                1740

ctg gtc gac cgg gcg aat acc ggg att gac acc att ctt tcc atg gag   7252
Leu Val Asp Arg Ala Asn Thr Gly Ile Asp Thr Ile Leu Ser Met Glu
   1745                1750                1755

acc cag agg ctt acc gaa ccc gcc ctg gaa gag ggg agt gat gtg ttt   7300
Thr Gln Arg Leu Thr Glu Pro Ala Leu Glu Glu Gly Ser Asp Val Phe
1760                1765                1770                1775

atg gac ttc tcc gga gcc aat gcc ctc tat ttc tgg gag ctg ttc tat   7348
Met Asp Phe Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe Tyr
             1780                1785                1790

tac acg ccg atg atg gtg ttc cag cgg ttg ttg cag gaa cag cac ttc   7396
Tyr Thr Pro Met Met Val Phe Gln Arg Leu Leu Gln Glu Gln His Phe
          1795                1800                1805

ccg gaa gcc acc cgc tgg ctg cag tat gtc tgg aac ccg gcc ggg cac   7444
Pro Glu Ala Thr Arg Trp Leu Gln Tyr Val Trp Asn Pro Ala Gly His
       1810                1815                1820

gtg gta aac ggg gtg ctg cag aat tac acc tgg aat gtc cgt ccg ctg   7492
Val Val Asn Gly Val Leu Gln Asn Tyr Thr Trp Asn Val Arg Pro Leu
   1825                1830                1835

gag gag gac acc ggc tgg aac gac tcg ccg ctg gac tcc att gac ccc   7540
Glu Glu Asp Thr Gly Trp Asn Asp Ser Pro Leu Asp Ser Ile Asp Pro
1840                1845                1850                1855

gat gca ata gcc cag tac gac ccc atg cat tac aag gtc gcc acc ttt   7588
Asp Ala Ile Ala Gln Tyr Asp Pro Met His Tyr Lys Val Ala Thr Phe
             1860                1865                1870

atg tcg tac ctc gac ctg ctg att gcc cgc ggt gat gcc gcc tac cgg   7636
Met Ser Tyr Leu Asp Leu Leu Ile Ala Arg Gly Asp Ala Ala Tyr Arg
          1875                1880                1885

ctg ctc gag cgg gac acc ctt aac gag gcc cgg atg tgg tac gtc cag   7684
Leu Leu Glu Arg Asp Thr Leu Asn Glu Ala Arg Met Trp Tyr Val Gln
       1890                1895                1900

gcc ctg aac ctt ctg ggc gac gag ccc tat att tcc ttt gac gcc gac   7732
Ala Leu Asn Leu Leu Gly Asp Glu Pro Tyr Ile Ser Phe Asp Ala Asp
   1905                1910                1915

tgg tcg gcg ttg acc ctg ggt gac gca gcc agc gag gtg acg cga cgc   7780
Trp Ser Ala Leu Thr Leu Gly Asp Ala Ala Ser Glu Val Thr Arg Arg
1920                1925                1930                1935

gat tac cag gag gcc ctg ctg gcc gtg cgc cgg ttg gtg ccc gct ccc   7828
Asp Tyr Gln Glu Ala Leu Leu Ala Val Arg Arg Leu Val Pro Ala Pro
             1940                1945                1950
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gag aca cgg acg gcg aat tcc ctg acg gca ctg ttc ctc ccg cag cag   7876
Glu Thr Arg Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Gln
          1955                1960                1965

aac gag gtg ctc aaa ggc tac tgg caa acc ttg gca cag cgg ctc cat   7924
Asn Glu Val Leu Lys Gly Tyr Trp Gln Thr Leu Ala Gln Arg Leu His
      1970                1975                1980

aac ctg cgc cac aac ctc tcc att gac ggc cag ccg ctt tcc ctg tcc   7972
Asn Leu Arg His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Ser
   1985                1990                1995

gtc tac gcc acg ccg tcc gaa ccg tcc gcc ctg cag agt gcc gtc gtc   8020
Val Tyr Ala Thr Pro Ser Glu Pro Ser Ala Leu Gln Ser Ala Val Val
2000                2005                2010                2015

aac agc gcg cag ggt gct gca gca ctg ccg gcc gcg gtg atg ccg ctt   8068
Asn Ser Ala Gln Gly Ala Ala Ala Leu Pro Ala Ala Val Met Pro Leu
             2020                2025                2030

tac agt ttc ccg gtc atg ctg gag aac gcc cgg ggg atg gtg agc ctg   8116
Tyr Ser Phe Pro Val Met Leu Glu Asn Ala Arg Gly Met Val Ser Leu
          2035                2040                2045

ctg acc ggg ttc ggc aac aca ctg ctc ggt att acc gag cgt cag gat   8164
Leu Thr Gly Phe Gly Asn Thr Leu Leu Gly Ile Thr Glu Arg Gln Asp
      2050                2055                2060

gcg gag gcg ctg gcc aaa ctg ctg cag acc cag ggc agt gaa ctg ata   8212
Ala Glu Ala Leu Ala Lys Leu Leu Gln Thr Gln Gly Ser Glu Leu Ile
   2065                2070                2075

cgc cag ggc ctt cgc cag cag gat aac gtc ctc gag gaa atc gat gcg   8260
Arg Gln Gly Leu Arg Gln Gln Asp Asn Val Leu Glu Glu Ile Asp Ala
2080                2085                2090                2095

gat att gcc gcc ctg gag gag agc cgc cgc ggc gcg cag atg cgt ttt   8308
Asp Ile Ala Ala Leu Glu Glu Ser Arg Arg Gly Ala Gln Met Arg Phe
             2100                2105                2110

gaa cgt tac aaa gtg ttg tac gag gcg gac gtc aac acc ggc gaa aaa   8356
Glu Arg Tyr Lys Val Leu Tyr Glu Ala Asp Val Asn Thr Gly Glu Lys
          2115                2120                2125

cag gcc atg gac ttg tac ctc agt tcg tcc gtg ctg tcg gca tca acc   8404
Gln Ala Met Asp Leu Tyr Leu Ser Ser Ser Val Leu Ser Ala Ser Thr
      2130                2135                2140

gcc gcg ctc ttt ttg gcc gag gcc gcg gcc gat atg ctg ccc aat att   8452
Ala Ala Leu Phe Leu Ala Glu Ala Ala Ala Asp Met Leu Pro Asn Ile
   2145                2150                2155

tac ggg ctg gcc gtc ggg ggc tcc cgc tat ggg gca cta ttt aaa gcc   8500
Tyr Gly Leu Ala Val Gly Gly Ser Arg Tyr Gly Ala Leu Phe Lys Ala
2160                2165                2170                2175

acc gcc atc ggc atc cag gtg tcc tcc gat gcc acc cgc ata tca gcg   8548
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Thr Ala Ile Gly Ile Gln Val Ser Ser Asp Ala Thr Arg Ile Ser Ala
                2180                2185                2190

gac aaa atc agc cag tcg gaa gtg tac cgc cgt cgc cgg gag gag tgg   8596
Asp Lys Ile Ser Gln Ser Glu Val Tyr Arg Arg Arg Arg Glu Glu Trp
           2195                2200                2205

gaa atc cag cgt gat agt gcg cag tct gac gtg gcg cag att gat gcc   8644
Glu Ile Gln Arg Asp Ser Ala Gln Ser Asp Val Ala Gln Ile Asp Ala
       2210                2215                2220

cag ctg gcg gcc atg gca gtg cgc cgg gaa ggg gct gag ctg cag aaa   8692
Gln Leu Ala Ala Met Ala Val Arg Arg Glu Gly Ala Glu Leu Gln Lys
   2225                2230                2235

act tac ctt gag acc cag cag acc cag gca cag gcg cag ttg gca ttc   8740
Thr Tyr Leu Glu Thr Gln Gln Thr Gln Ala Gln Ala Gln Leu Ala Phe
2240                2245                2250                2255

ctg cag agt aag ttc aac aat acg gct ctg tac agc tgg ctg cgg ggc   8788
Leu Gln Ser Lys Phe Asn Asn Thr Ala Leu Tyr Ser Trp Leu Arg Gly
                2260                2265                2270

agg ttg tcc gcc att tat tac cag ttc tat gac ctg gca gta tcc cgc   8836
Arg Leu Ser Ala Ile Tyr Tyr Gln Phe Tyr Asp Leu Ala Val Ser Arg
           2275                2280                2285

tgc ctg atg gcg caa cag gcc tgg cag tgg gat aaa ttc gag act agg   8884
Cys Leu Met Ala Gln Gln Ala Trp Gln Trp Asp Lys Phe Glu Thr Arg
       2290                2295                2300

tcg ttt atc cag ccg ggg gcc tgg atg ggg gca aat gcc ggt ctg ctg   8932
Ser Phe Ile Gln Pro Gly Ala Trp Met Gly Ala Asn Ala Gly Leu Leu
   2305                2310                2315

gcc ggg gaa acc ctg atg ctg aat ctg gcg cag atg gag cag gcc tgg   8980
Ala Gly Glu Thr Leu Met Leu Asn Leu Ala Gln Met Glu Gln Ala Trp
2320                2325                2330                2335

ctg acg ggg gat gag cgg gca ata gag gtg acg cgg acg gtc tgc ctg   9028
Leu Thr Gly Asp Glu Arg Ala Ile Glu Val Thr Arg Thr Val Cys Leu
                2340                2345                2350

tcg gag gtc tat acc agc ctc gcg gag gat gcg gca ttc tct ctg gcc   9076
Ser Glu Val Tyr Thr Ser Leu Ala Glu Asp Ala Ala Phe Ser Leu Ala
           2355                2360                2365

gac aag gtg gtg gaa ctg gtc agt aac ggt tcg ggc agt gcg ggt acg   9124
Asp Lys Val Val Glu Leu Val Ser Asn Gly Ser Gly Ser Ala Gly Thr
       2370                2375                2380

aaa agc aac gga tta cag atg gat caa cag caa ctc gag gcc acc ctg   9172
Lys Ser Asn Gly Leu Gln Met Asp Gln Gln Gln Leu Glu Ala Thr Leu
   2385                2390                2395

aaa ctg gct gac ctc ggt atc ggc aac gat tac ccg gtc tcc ctt ggc   9220
Lys Leu Ala Asp Leu Gly Ile Gly Asn Asp Tyr Pro Val Ser Leu Gly
2400                2405                2410                2415
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acc atg agg cgc atc aaa caa ata agc gtc acg ctc ccg gcg ctg gtc   9268
Thr Met Arg Arg Ile Lys Gln Ile Ser Val Thr Leu Pro Ala Leu Val
              2420                2425                2430

ggc ccc tat cag gac gtc cgt gcg gtt ctc agc tac ggc gga agt atg   9316
Gly Pro Tyr Gln Asp Val Arg Ala Val Leu Ser Tyr Gly Gly Ser Met
          2435                2440                2445

gtc atg ccc cgg ggt tgc agc gcg ctg gcg gtc tca cac gga atg aac   9364
Val Met Pro Arg Gly Cys Ser Ala Leu Ala Val Ser His Gly Met Asn
       2450                2455                2460

gac agc ggc caa ttc caa ctg gat ttc aat gac ccg cgt tac ctg ccg   9412
Asp Ser Gly Gln Phe Gln Leu Asp Phe Asn Asp Pro Arg Tyr Leu Pro
   2465                2470                2475

ttt gaa gga ctt cca gtt gat gac aca ggg acc ctg aca ctg agc ttc   9460
Phe Glu Gly Leu Pro Val Asp Asp Thr Gly Thr Leu Thr Leu Ser Phe
2480                2485                2490                2495

ccg gat gct gac ggc aaa caa cag gcg atg ctc ctc agt ctg agc gac   9508
Pro Asp Ala Asp Gly Lys Gln Gln Ala Met Leu Leu Ser Leu Ser Asp
              2500                2505                2510

atc atc ctg cat atc cgt tac acc att atc agc tga tag gtatcaacat    9557
Ile Ile Leu His Ile Arg Tyr Thr Ile Ile Ser
          2515                2520

agcgcaggcc cccgaacgag ggcctgcgag gagactgagc atg caa aat cat caa   9612
                                          Met Gln Asn His Gln
                                       2525

gac atg gcc att act gcc ccc acg ttg cct tcc ggg ggc ggt gcg gtc   9660
Asp Met Ala Ile Thr Ala Pro Thr Leu Pro Ser Gly Gly Gly Ala Val
2530                2535                2540                2545

acc ggg ctc aag ggt gat atc gcg gcg gca ggg ccg gat ggt gcg gcg   9708
Thr Gly Leu Lys Gly Asp Ile Ala Ala Ala Gly Pro Asp Gly Ala Ala
              2550                2555                2560

acc ctg agt att ccc ttg ccg gtt agc ccc ggt cgg ggt tac gcc ccc   9756
Thr Leu Ser Ile Pro Leu Pro Val Ser Pro Gly Arg Gly Tyr Ala Pro
          2565                2570                2575

act ggg gca ctt aat tat cac agc cgg tcg ggg aac ggc ccc ttt ggc   9804
Thr Gly Ala Leu Asn Tyr His Ser Arg Ser Gly Asn Gly Pro Phe Gly
       2580                2585                2590

att ggc tgg ggt atc ggc ggt gct gct gtc cag cgt cgt acg cgc aac   9852
Ile Gly Trp Gly Ile Gly Gly Ala Ala Val Gln Arg Arg Thr Arg Asn
   2595                2600                2605

gga gca cct acc tac gat gat act gat gaa ttc acc ggt ccg gac ggt   9900
Gly Ala Pro Thr Tyr Asp Asp Thr Asp Glu Phe Thr Gly Pro Asp Gly
2610                2615                2620                2625

gag gtg ctg gtg ccg gca ctc acg gct gct ggc acc caa gaa gca cgg   9948
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Glu Val Leu Val Pro Ala Leu Thr Ala Ala Gly Thr Gln Glu Ala Arg
                2630                2635                2640

cag gcc acc tca cta ctg ggg ata aac cca ggc gga agc ttc aac gtt   9996
Gln Ala Thr Ser Leu Leu Gly Ile Asn Pro Gly Gly Ser Phe Asn Val
            2645                2650                2655

cag gtt tac cgt tca cgt acg gag ggt agt ctc agc cgc ctt gag cgt   10044
Gln Val Tyr Arg Ser Arg Thr Glu Gly Ser Leu Ser Arg Leu Glu Arg
        2660                2665                2670

tgg ctg ccc gcc gac gag aca gaa acg gaa ttt tgg gtg tta tat acc   10092
Trp Leu Pro Ala Asp Glu Thr Glu Thr Glu Phe Trp Val Leu Tyr Thr
    2675                2680                2685

cct gac gga cag gtg gct ctg ctg ggc cga aat gcg cag gct cgc atc   10140
Pro Asp Gly Gln Val Ala Leu Leu Gly Arg Asn Ala Gln Ala Arg Ile
2690                2695                2700                2705

agc aac ccc aca gcc cca aca cag acg gcg gtt tgg ctg atg gag tcc   10188
Ser Asn Pro Thr Ala Pro Thr Gln Thr Ala Val Trp Leu Met Glu Ser
                2710                2715                2720

tcg gta tca ctt acc ggc gaa cag atg tat tac caa tac cgt gcg gaa   10236
Ser Val Ser Leu Thr Gly Glu Gln Met Tyr Tyr Gln Tyr Arg Ala Glu
            2725                2730                2735

gat gat gac ggt tgt gac gag gcg gag cgc gac gcg cac ccg cag gcc   10284
Asp Asp Asp Gly Cys Asp Glu Ala Glu Arg Asp Ala His Pro Gln Ala
        2740                2745                2750

ggc gcc caa cgt tat ccg gtg gcg gtc tgg tat ggt aac cgt cag gcg   10332
Gly Ala Gln Arg Tyr Pro Val Ala Val Trp Tyr Gly Asn Arg Gln Ala
    2755                2760                2765

gct cgg acg cta ccg gcg ctg gtg tcg aca cca tca atg gat agc tgg   10380
Ala Arg Thr Leu Pro Ala Leu Val Ser Thr Pro Ser Met Asp Ser Trp
2770                2775                2780                2785

ctg ttt atc ctg gtg ttt gat tat ggt gag cgt agc tcg gtg ctg tct   10428
Leu Phe Ile Leu Val Phe Asp Tyr Gly Glu Arg Ser Ser Val Leu Ser
                2790                2795                2800

gaa gcg ccg gcc tgg caa aca cca gga agt ggg gag tgg ctg tgt cgt   10476
Glu Ala Pro Ala Trp Gln Thr Pro Gly Ser Gly Glu Trp Leu Cys Arg
            2805                2810                2815

cag gat tgt ttt tcc ggg tat gag ttt ggt ttt aac ctg cgg act cgc   10524
Gln Asp Cys Phe Ser Gly Tyr Glu Phe Gly Phe Asn Leu Arg Thr Arg
        2820                2825                2830

cgc ctg tgc cgt cag gtt ttg atg ttc cat tac cta ggt gtt ctg gcg   10572
Arg Leu Cys Arg Gln Val Leu Met Phe His Tyr Leu Gly Val Leu Ala
    2835                2840                2845

ggg agt tcg gga gcg aat gat gcg cca gca ttg att tct cgc ctg ttg   10620
Gly Ser Ser Gly Ala Asn Asp Ala Pro Ala Leu Ile Ser Arg Leu Leu
2850                2855                2860                2865
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ctg gac tac agg gaa agt cct tca ctc agt ctg ctc gag aac gtg cac   10668
Leu Asp Tyr Arg Glu Ser Pro Ser Leu Ser Leu Leu Glu Asn Val His
                2870                2875                2880

cag gtg gct tat gag tcg gac ggg acg tct tgt gcc ttg ccg gca ctg   10716
Gln Val Ala Tyr Glu Ser Asp Gly Thr Ser Cys Ala Leu Pro Ala Leu
            2885                2890                2895

gca ttg ggg tgg caa acc ttt acc ccg ccg aca ttg tcg gca tgg cag   10764
Ala Leu Gly Trp Gln Thr Phe Thr Pro Pro Thr Leu Ser Ala Trp Gln
        2900                2905                2910

acg cgt gac gat atg ggc aag ttg agt ttg ctt caa ccc tat cag ctt   10812
Thr Arg Asp Asp Met Gly Lys Leu Ser Leu Leu Gln Pro Tyr Gln Leu
    2915                2920                2925

gta gac ctt aac ggc gaa ggt gtg gtg ggt atc ctg tat cag gac agc   10860
Val Asp Leu Asn Gly Glu Gly Val Val Gly Ile Leu Tyr Gln Asp Ser
2930                2935                2940                2945

ggt gcc tgg tgg tac cgt gaa ccg gta cgc cag tcg ggg gat gat ccg   10908
Gly Ala Trp Trp Tyr Arg Glu Pro Val Arg Gln Ser Gly Asp Asp Pro
                2950                2955                2960

gat gct gtg acc tgg ggg gcg gct gcg gcc ctg ccg aca atg ccc gct   10956
Asp Ala Val Thr Trp Gly Ala Ala Ala Ala Leu Pro Thr Met Pro Ala
            2965                2970                2975

ttg cat aac agc ggc atc ctg gcg gat ctt aat ggg gat ggt cgg ctg   11004
Leu His Asn Ser Gly Ile Leu Ala Asp Leu Asn Gly Asp Gly Arg Leu
        2980                2985                2990

gag tgg gtc gtt acc gcc ccc ggt gtg gcg ggg atg tat gat cgc acc   11052
Glu Trp Val Val Thr Ala Pro Gly Val Ala Gly Met Tyr Asp Arg Thr
    2995                3000                3005

ccc ggc cgc gac tgg ttg cat ttc acc ccc ctg tca gcc ttg ccc gta   11100
Pro Gly Arg Asp Trp Leu His Phe Thr Pro Leu Ser Ala Leu Pro Val
3010                3015                3020                3025

gaa tat gcg cat cca aaa gca gtg ctc gcc gat atc ctg ggg gct ggg   11148
Glu Tyr Ala His Pro Lys Ala Val Leu Ala Asp Ile Leu Gly Ala Gly
                3030                3035                3040

tta acg gac atg gtg ctt atc ggg ccg cgc agt gtt cgc ctc tat tcc   11196
Leu Thr Asp Met Val Leu Ile Gly Pro Arg Ser Val Arg Leu Tyr Ser
            3045                3050                3055

ggc aaa aac gat ggt tgg aat aaa ggg gag acc gtg cag caa acg gaa   11244
Gly Lys Asn Asp Gly Trp Asn Lys Gly Glu Thr Val Gln Gln Thr Glu
        3060                3065                3070

aga ctc act ctg ccg gtc ccg ggg gtt gac cca cgt acc ctc gtg gcg   11292
Arg Leu Thr Leu Pro Val Pro Gly Val Asp Pro Arg Thr Leu Val Ala
    3075                3080                3085

ttc agt gat atg gct ggc agt gga cag cag cat ttg acg gag gtg cgt   11340
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Phe Ser Asp Met Ala Gly Ser Gly Gln Gln His Leu Thr Glu Val Arg
3090                3095                3100                3105

gct aat gga gta cgt tac tgg cca aac ctg ggg cac ggt cgt ttc ggt   11388
Ala Asn Gly Val Arg Tyr Trp Pro Asn Leu Gly His Gly Arg Phe Gly
                3110                3115                3120

cag ccg gtg aat att ccc ggt ttt agc cag tca gtg act acg ttt aac   11436
Gln Pro Val Asn Ile Pro Gly Phe Ser Gln Ser Val Thr Thr Phe Asn
            3125                3130                3135

cct gac cag ata ttg ctg gcc gat acc gac ggt tcc ggt acc acg gac   11484
Pro Asp Gln Ile Leu Leu Ala Asp Thr Asp Gly Ser Gly Thr Thr Asp
        3140                3145                3150

ctg att tat gcg atg agt gac cgg tta gtc att tat ttc aac cag agt   11532
Leu Ile Tyr Ala Met Ser Asp Arg Leu Val Ile Tyr Phe Asn Gln Ser
    3155                3160                3165

ggt aat tat ttc gcc gag ccg cat acg ctg ctc ttg ccg aaa ggt gtg   11580
Gly Asn Tyr Phe Ala Glu Pro His Thr Leu Leu Leu Pro Lys Gly Val
3170                3175                3180                3185

cgc tat gat cgc acc tgc agt ctg caa gtg gcg gat atc cag ggg ctg   11628
Arg Tyr Asp Arg Thr Cys Ser Leu Gln Val Ala Asp Ile Gln Gly Leu
                3190                3195                3200

ggg gtg cct agc ctg tta ctg acg gtc ccc cat gtc gcg cct cat cac   11676
Gly Val Pro Ser Leu Leu Leu Thr Val Pro His Val Ala Pro His His
            3205                3210                3215

tgg gtg tgc cat tta tcg gca gac aaa ccc tgg ttg ttg aat ggc atg   11724
Trp Val Cys His Leu Ser Ala Asp Lys Pro Trp Leu Leu Asn Gly Met
        3220                3225                3230

aac aac aat atg ggg gcc cgg cat gca ctg cac tat cgc agt tcg gtg   11772
Asn Asn Asn Met Gly Ala Arg His Ala Leu His Tyr Arg Ser Ser Val
    3235                3240                3245

cag ttc tgg ctg gat gag aaa gcc gag gca ctg gcg gca ggc agt tcc   11820
Gln Phe Trp Leu Asp Glu Lys Ala Glu Ala Leu Ala Ala Gly Ser Ser
3250                3255                3260                3265

cct gcc tgc tac ctg cca ttt aca ttg cat acc ctg tgg cgt tcg gtg   11868
Pro Ala Cys Tyr Leu Pro Phe Thr Leu His Thr Leu Trp Arg Ser Val
                3270                3275                3280

gtg cag gat gag atc acc ggt aac cgt ctg gtc agc gac gtg ctt tat   11916
Val Gln Asp Glu Ile Thr Gly Asn Arg Leu Val Ser Asp Val Leu Tyr
            3285                3290                3295

cgc cac ggc gtc tgg gac ggg cag gaa cgc gag ttt cgg ggg ttt ggt   11964
Arg His Gly Val Trp Asp Gly Gln Glu Arg Glu Phe Arg Gly Phe Gly
        3300                3305                3310

ttt gtt gag atc agg gat acc gat acc ttg gca agc cag ggt acg gcg   12012
Phe Val Glu Ile Arg Asp Thr Asp Thr Leu Ala Ser Gln Gly Thr Ala
    3315                3320                3325
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acg gaa ctg agt atg cct tct gtg agc cgg aac tgg tat gcc acc ggg   12060
Thr Glu Leu Ser Met Pro Ser Val Ser Arg Asn Trp Tyr Ala Thr Gly
3330                3335                3340                3345

gta ccg gca gta gac gag cgt ctg ccg gag acg tat tgg caa aac gat   12108
Val Pro Ala Val Asp Glu Arg Leu Pro Glu Thr Tyr Trp Gln Asn Asp
                3350                3355                3360

gcc gcc gct ttt gcc gat ttc gcg acc cgt ttc act gtc ggt tca gga   12156
Ala Ala Ala Phe Ala Asp Phe Ala Thr Arg Phe Thr Val Gly Ser Gly
            3365                3370                3375

gag gat gag cag aca tat act ccg gac gac agc aag aca ttc tgg ttg   12204
Glu Asp Glu Gln Thr Tyr Thr Pro Asp Asp Ser Lys Thr Phe Trp Leu
        3380                3385                3390

cag cga gcc ctg aaa ggc atc ctg ctg cgc agt gag tta tac ggt gcc   12252
Gln Arg Ala Leu Lys Gly Ile Leu Leu Arg Ser Glu Leu Tyr Gly Ala
    3395                3400                3405

gat ggc agc agc cag gcc gat atc cct tac agc gtc act gag tct cgc   12300
Asp Gly Ser Ser Gln Ala Asp Ile Pro Tyr Ser Val Thr Glu Ser Arg
3410                3415                3420                3425

ccg cag gta cgg cta gtt gaa gcg aat gga gac tac ccg gtg gtg tgg   12348
Pro Gln Val Arg Leu Val Glu Ala Asn Gly Asp Tyr Pro Val Val Trp
                3430                3435                3440

ccg atg ggc gcg gaa agc cgt acg tca gtt tat gaa cgg tac cac aat   12396
Pro Met Gly Ala Glu Ser Arg Thr Ser Val Tyr Glu Arg Tyr His Asn
            3445                3450                3455

gat cct caa tgc caa cag cag gcg gta ctc ctc agt gat gaa tac ggt   12444
Asp Pro Gln Cys Gln Gln Gln Ala Val Leu Leu Ser Asp Glu Tyr Gly
        3460                3465                3470

ttc cca ctg cgt cag gtc agt gtc aat tat cca cga cgc cct ccg tcg   12492
Phe Pro Leu Arg Gln Val Ser Val Asn Tyr Pro Arg Arg Pro Pro Ser
    3475                3480                3485

gcg gac aat cca tat ccg gcg tcc tta ccg gcg acg ctg ttc gcc aac   12540
Ala Asp Asn Pro Tyr Pro Ala Ser Leu Pro Ala Thr Leu Phe Ala Asn
3490                3495                3500                3505

agt tat gac gag cag cag cag ata tta cgc ctg ggg ttg caa cag agc   12588
Ser Tyr Asp Glu Gln Gln Gln Ile Leu Arg Leu Gly Leu Gln Gln Ser
                3510                3515                3520

agt gca cat cac ctt gtt tca ctg tct gag ggg cat tgg ttg ttg ggg   12636
Ser Ala His His Leu Val Ser Leu Ser Glu Gly His Trp Leu Leu Gly
            3525                3530                3535

ttg gcg gag gcg tcg cgg gac gat gta ttc acg tac tct gcg gac aac   12684
Leu Ala Glu Ala Ser Arg Asp Asp Val Phe Thr Tyr Ser Ala Asp Asn
        3540                3545                3550

gtg ccg gaa ggg ggt ctg acg ctg gaa cac ctg ttg gcg ccc gaa agc   12732
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Val Pro Glu Gly Gly Leu Thr Leu Glu His Leu Leu Ala Pro Glu Ser
   3555                3560                3565

ctg gtc tcg gat agt cag gtc ggt acg ctg gcg ggt cag cag caa gtc   12780
Leu Val Ser Asp Ser Gln Val Gly Thr Leu Ala Gly Gln Gln Gln Val
3570                3575                3580                3585

tgg tat ctg gat tca caa gac gtt gcc acc gtc gct gct ccg cca ctc   12828
Trp Tyr Leu Asp Ser Gln Asp Val Ala Thr Val Ala Ala Pro Pro Leu
                3590                3595                3600

ccc ccc aag gta gct ttt atc gaa acg gcc gtg ctg gat gag ggt atg   12876
Pro Pro Lys Val Ala Phe Ile Glu Thr Ala Val Leu Asp Glu Gly Met
           3605                3610                3615

gtc agt tca ctg gct gcc tac att gtg gat gaa cat ctc gag caa gcc   12924
Val Ser Ser Leu Ala Ala Tyr Ile Val Asp Glu His Leu Glu Gln Ala
       3620                3625                3630

ggt tac cgg caa tcc gga tac ctt ttc cct cga ggc agg gaa gca gaa   12972
Gly Tyr Arg Gln Ser Gly Tyr Leu Phe Pro Arg Gly Arg Glu Ala Glu
   3635                3640                3645

cag gca ttg tgg acc cag tgt cag gga tat gtt acc tat gcc ggc gca   13020
Gln Ala Leu Trp Thr Gln Cys Gln Gly Tyr Val Thr Tyr Ala Gly Ala
3650                3655                3660                3665

gag cat ttc tgg cta ccg cta tcc ttt cgg gac agt atg ttg acc ggc   13068
Glu His Phe Trp Leu Pro Leu Ser Phe Arg Asp Ser Met Leu Thr Gly
                3670                3675                3680

cca gtt acc gtg acg cgt gac gcg tac gac tgc gtc atc acg cag tgg   13116
Pro Val Thr Val Thr Arg Asp Ala Tyr Asp Cys Val Ile Thr Gln Trp
           3685                3690                3695

cag gat gcc gca ggg att gtc acc aca gcc gac tat gac tgg cgc ttc   13164
Gln Asp Ala Ala Gly Ile Val Thr Thr Ala Asp Tyr Asp Trp Arg Phe
       3700                3705                3710

ctg acg ccc gtc cgg gtg acg gac ccc aat gat aat ctg cag tcc gtc   13212
Leu Thr Pro Val Arg Val Thr Asp Pro Asn Asp Asn Leu Gln Ser Val
   3715                3720                3725

act ctg gat gct ctg ggc cgg gtg acc acc ctg cga ttc tgg ggc acg   13260
Thr Leu Asp Ala Leu Gly Arg Val Thr Thr Leu Arg Phe Trp Gly Thr
3730                3735                3740                3745

gag aat ggt att gcc acc ggt tac agt gat gcc acg ttg tcc gtt ccg   13308
Glu Asn Gly Ile Ala Thr Gly Tyr Ser Asp Ala Thr Leu Ser Val Pro
                3750                3755                3760

gac ggc gca gca gcc gct ctg gcg ttg acg gcg ccc cta cca gta gca   13356
Asp Gly Ala Ala Ala Ala Leu Ala Leu Thr Ala Pro Leu Pro Val Ala
           3765                3770                3775

cag tgt ctg gtg tat gtc acg gac agt tgg gga gat gac gac aat gag   13404
Gln Cys Leu Val Tyr Val Thr Asp Ser Trp Gly Asp Asp Asp Asn Glu
       3780                3785                3790
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aaa atg ccc ccg cac gtg gtc gtg ctg gct acc gat cgc tat gac agt   13452
Lys Met Pro Pro His Val Val Val Leu Ala Thr Asp Arg Tyr Asp Ser
   3795                3800                3805

gat acc gga cag cag gtc cgc caa cag gtg aca ttc agt gac ggt ttt   13500
Asp Thr Gly Gln Gln Val Arg Gln Gln Val Thr Phe Ser Asp Gly Phe
3810                3815                3820                3825

ggg cgt gag ttg caa tcg gca acc cgg cag gcc gag ggc aac gcc tgg   13548
Gly Arg Glu Leu Gln Ser Ala Thr Arg Gln Ala Glu Gly Asn Ala Trp
               3830                3835                3840

caa cga gga cgc gac ggc aaa ctg gtg acg gcc agt gac gga ttg ccg   13596
Gln Arg Gly Arg Asp Gly Lys Leu Val Thr Ala Ser Asp Gly Leu Pro
          3845                3850                3855

gtc act gta gca acg aat ttc cgc tgg gcg gtc acc ggg agg gcg gag   13644
Val Thr Val Ala Thr Asn Phe Arg Trp Ala Val Thr Gly Arg Ala Glu
       3860                3865                3870

tat gac aat aaa ggt ctg cct gtt cgg gtt tat cag ccg tat ttt ctg   13692
Tyr Asp Asn Lys Gly Leu Pro Val Arg Val Tyr Gln Pro Tyr Phe Leu
   3875                3880                3885

gac agt tgg caa tat gtc agt gat gac agt gcc cgc cag gac ctg tat   13740
Asp Ser Trp Gln Tyr Val Ser Asp Asp Ser Ala Arg Gln Asp Leu Tyr
3890                3895                3900                3905

gcc gac acg cac ttt tac gat ccg acg gca cgg gaa tgg cag gtt att   13788
Ala Asp Thr His Phe Tyr Asp Pro Thr Ala Arg Glu Trp Gln Val Ile
               3910                3915                3920

acg gca aaa ggt gaa cgg cga cag gtg ctg tat acc ccg tgg ttt gtg   13836
Thr Ala Lys Gly Glu Arg Arg Gln Val Leu Tyr Thr Pro Trp Phe Val
          3925                3930                3935

gtc agt gaa gac gag aat gat acc gtt ggg cta aac gac gca tcc tga   13884
Val Ser Glu Asp Glu Asn Asp Thr Val Gly Leu Asn Asp Ala Ser
       3940                3945                3950

ctgggaagga ggggggacg gtg atg agt ccg tcg ccc ctg aca ggc gct gcc 13937
                        Met Ser Pro Ser Pro Leu Thr Gly Ala Ala
                           3955                3960

ctg atg gag aca aag atg aaa ata cac tat cag gtt gcg gcg gtt gtg   13985
Leu Met Glu Thr Lys Met Lys Ile His Tyr Gln Val Ala Ala Val Val
   3965                3970                3975

ctg aca ggt gtt atg gtt tgg ggg ctt tcc cat tgg cgt tac acc gtc   14033
Leu Thr Gly Val Met Val Trp Gly Leu Ser His Trp Arg Tyr Thr Val
3980                3985                3990                3995

ggt tac cac gcg gca gat act caa tgg caa caa cgc cag gcc gaa cag   14081
Gly Tyr His Ala Ala Asp Thr Gln Trp Gln Gln Arg Gln Ala Glu Gln
               4000                4005                4010

gaa agg gcc gat gcg ttg gcc ctc ctg gca gca gaa acc cgg gaa aga   14129
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Glu Arg Ala Asp Ala Leu Ala Leu Leu Ala Ala Glu Thr Arg Glu Arg
          4015                4020                4025

aag tgg gag cag caa cga cag act gac atg aac aag gtg gct ata cat   14177
Lys Trp Glu Gln Gln Arg Gln Thr Asp Met Asn Lys Val Ala Ile His
       4030                4035                4040

gct gaa gaa gaa ctg gct gct gcg cgt gac gct gcc gct gat gct cag   14225
Ala Glu Glu Glu Leu Ala Ala Ala Arg Asp Ala Ala Ala Asp Ala Gln
   4045                4050                4055

cgc act ggt cag cgc ctg cag cac acc gtt acc acc ctc cag cgg caa   14273
Arg Thr Gly Gln Arg Leu Gln His Thr Val Thr Thr Leu Gln Arg Gln
4060                4065                4070                4075

ctt gcc agt cgt gaa acc cgc cgc ctt tcc gca gct acc gct atc ggt   14321
Leu Ala Ser Arg Glu Thr Arg Arg Leu Ser Ala Ala Thr Ala Ile Gly
               4080                4085                4090

aca gac gac ctc gga ggc caa ccc ggc gtt ttg ttt gcc gaa ctg ttc   14369
Thr Asp Asp Leu Gly Gly Gln Pro Gly Val Leu Phe Ala Glu Leu Phe
          4095                4100                4105

cgc cgc gct gac cag aga gcg gga gag ctg gca gcg tat gct gac agg   14417
Arg Arg Ala Asp Gln Arg Ala Gly Glu Leu Ala Ala Tyr Ala Asp Arg
       4110                4115                4120

acc aga gtg aaa tgg cag gcc tgc ggg cgc gcc tat cag gcg gct acg   14465
Thr Arg Val Lys Trp Gln Ala Cys Gly Arg Ala Tyr Gln Ala Ala Thr
   4125                4130                4135

cac gaa gca gaa aaa taa ggcgatttag ccgttaagga aaagtgacgg          14513
His Glu Ala Glu Lys
4140                4145

tgttttcgcg attaatatta acaggagatc ac atg agc aca tcc ttg ttc agt   14566
                                  Met Ser Thr Ser Leu Phe Ser
                                                  4150

agc acc ccg tcg gtc gcg gtg ctc gac aac cgc ggc ctg ttg gtg cgg   14614
Ser Thr Pro Ser Val Ala Val Leu Asp Asn Arg Gly Leu Leu Val Arg
       4155                4160                4165

gag ctg cag tac tac cgc cat ccg gat aca ccg gag gag acg gac gag   14662
Glu Leu Gln Tyr Tyr Arg His Pro Asp Thr Pro Glu Glu Thr Asp Glu
   4170                4175                4180

cgt atc acc tgc cat cag cac gat gag cgc ggc agc ttg tca caa agc   14710
Arg Ile Thr Cys His Gln His Asp Glu Arg Gly Ser Leu Ser Gln Ser
4185                4190                4195                4200

gcc gac ccg cgg tta cac gcg gcc ggt ctg aca aat ttc acg tac ctg   14758
Ala Asp Pro Arg Leu His Ala Ala Gly Leu Thr Asn Phe Thr Tyr Leu
               4205                4210                4215

aat agc ctg acc ggg aca gta ctg cag agc gtc agc gcc gat gcc ggt   14806
Asn Ser Leu Thr Gly Thr Val Leu Gln Ser Val Ser Ala Asp Ala Gly
          4220                4225                4230
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acg tcg ctg gaa ctg agc gat gcc gcc ggg cgg gcg ttt ctg gcc gtc   14854
Thr Ser Leu Glu Leu Ser Asp Ala Ala Gly Arg Ala Phe Leu Ala Val
       4235                4240                4245

acc ggg gct ggg acg gaa gac gcg gtc acc cgc acc tgg caa tat gaa   14902
Thr Gly Ala Gly Thr Glu Asp Ala Val Thr Arg Thr Trp Gln Tyr Glu
   4250                4255                4260

gac gat acc ctg ccg ggc cgc ccg ctg agc atc acc gag cag gtt acc   14950
Asp Asp Thr Leu Pro Gly Arg Pro Leu Ser Ile Thr Glu Gln Val Thr
4265                4270                4275                4280

ggt gaa gcc gcc caa att acg gaa cgc ttc gtg tac gct ggc aat acg   14998
Gly Glu Ala Ala Gln Ile Thr Glu Arg Phe Val Tyr Ala Gly Asn Thr
                4285                4290                4295

gat gcc gag aag att ctc aat ctg gct ggc cag tgt gtc agt cat tac   15046
Asp Ala Glu Lys Ile Leu Asn Leu Ala Gly Gln Cys Val Ser His Tyr
           4300                4305                4310

gat acc gcc gga ctg gtg cag acg gac agc atc gcc ctg agc ggc gtg   15094
Asp Thr Ala Gly Leu Val Gln Thr Asp Ser Ile Ala Leu Ser Gly Val
       4315                4320                4325

ccg ctc gcc gtc acg cgg cag ttg ctg ccc gac gcg gcg ggg gcc aac   15142
Pro Leu Ala Val Thr Arg Gln Leu Leu Pro Asp Ala Ala Gly Ala Asn
   4330                4335                4340

tgg atg ggt gag gat gcc tcg gcc tgg aat gac ctg ctg gat ggg gag   15190
Trp Met Gly Glu Asp Ala Ser Ala Trp Asn Asp Leu Leu Asp Gly Glu
4345                4350                4355                4360

acg ttc ttc acc cag acc cac gct gat gcg acc ggc gcc gtc ctg agc   15238
Thr Phe Phe Thr Gln Thr His Ala Asp Ala Thr Gly Ala Val Leu Ser
                4365                4370                4375

atc acc gat gca aaa ggt aat ctg cag cgt gtg gca tat gat gtg gct   15286
Ile Thr Asp Ala Lys Gly Asn Leu Gln Arg Val Ala Tyr Asp Val Ala
           4380                4385                4390

ggg ctg cta tcg ggc agt tgg ttg acg ctg aag gac ggc acg gag cag   15334
Gly Leu Leu Ser Gly Ser Trp Leu Thr Leu Lys Asp Gly Thr Glu Gln
       4395                4400                4405

gtc atc gtg gcc tcc ctg acg tac tcg gcc gcc ggg aaa aag ttg cgt   15382
Val Ile Val Ala Ser Leu Thr Tyr Ser Ala Ala Gly Lys Lys Leu Arg
   4410                4415                4420

gaa gaa cac ggc aac ggc gtg gta acc tcg tat att tac gag ccg gaa   15430
Glu Glu His Gly Asn Gly Val Val Thr Ser Tyr Ile Tyr Glu Pro Glu
4425                4430                4435                4440

aca cag cgc ctg acg ggg att aaa acg gaa cgt ccg tct ggg cac gtt   15478
Thr Gln Arg Leu Thr Gly Ile Lys Thr Glu Arg Pro Ser Gly His Val
                4445                4450                4455

gcc gga gca aaa gtg ctg cag gac ctg cgc tat acg tat gac ccg gta   15526
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Ala Gly Ala Lys Val Leu Gln Asp Leu Arg Tyr Thr Tyr Asp Pro Val
          4460                4465                4470

ggc aac gta ctc agc gtc aat aac gat gcg gaa gag acc cgc ttc tgg   15574
Gly Asn Val Leu Ser Val Asn Asn Asp Ala Glu Glu Thr Arg Phe Trp
       4475                4480                4485

cgt aac cag aaa gtg gta ccg gag aat acg tac atc tac gac agc ctg   15622
Arg Asn Gln Lys Val Val Pro Glu Asn Thr Tyr Ile Tyr Asp Ser Leu
   4490                4495                4500

tac cag ctg gtc agc gcc aca ggg cgt gag atg gcc aat gcc ggc cag   15670
Tyr Gln Leu Val Ser Ala Thr Gly Arg Glu Met Ala Asn Ala Gly Gln
4505                4510                4515                4520

cag ggc aac gac tta cca tcc gct aca gcc ccc ctt cct aca gac agc   15718
Gln Gly Asn Asp Leu Pro Ser Ala Thr Ala Pro Leu Pro Thr Asp Ser
               4525                4530                4535

tct gcc tac acc aat tac acg cgc acc tac cgt tat gac cgt ggt ggc   15766
Ser Ala Tyr Thr Asn Tyr Thr Arg Thr Tyr Arg Tyr Asp Arg Gly Gly
          4540                4545                4550

aac ctg acg cag atg cgc cac agt gcc cct gcc acg aac aat aat tat   15814
Asn Leu Thr Gln Met Arg His Ser Ala Pro Ala Thr Asn Asn Asn Tyr
       4555                4560                4565

acg aca gac atc acg gtt agt gac cgc agc aat agg gcg gta ctg agc   15862
Thr Thr Asp Ile Thr Val Ser Asp Arg Ser Asn Arg Ala Val Leu Ser
   4570                4575                4580

acg ttg gcg gaa gtg ccg tca gat gtt gat atg ctg ttc agt gca gga   15910
Thr Leu Ala Glu Val Pro Ser Asp Val Asp Met Leu Phe Ser Ala Gly
4585                4590                4595                4600

ggt cac cag aag cac ctg cag ccg ggg caa gca ctg gtg tgg acg cca   15958
Gly His Gln Lys His Leu Gln Pro Gly Gln Ala Leu Val Trp Thr Pro
               4605                4610                4615

cgt gga gaa ctg caa aag gtg aca ccg gtg gtg cgt gat ggg ggg gcg   16006
Arg Gly Glu Leu Gln Lys Val Thr Pro Val Val Arg Asp Gly Gly Ala
          4620                4625                4630

gac gac agc gaa agc tat cgg tat gat gcg ggc agt cag cgt att atc   16054
Asp Asp Ser Glu Ser Tyr Arg Tyr Asp Ala Gly Ser Gln Arg Ile Ile
       4635                4640                4645

aaa acc ggc acg cgg caa act ggc aac aac gtt cag aca cag cgg gta   16102
Lys Thr Gly Thr Arg Gln Thr Gly Asn Asn Val Gln Thr Gln Arg Val
   4650                4655                4660

gtg tac ctg ccg ggg ctg gag tta cgt atc atg gca aat ggc gtg acg   16150
Val Tyr Leu Pro Gly Leu Glu Leu Arg Ile Met Ala Asn Gly Val Thr
4665                4670                4675                4680

gaa aaa gaa agc ctg cag gtt att acg gtg ggc gag gct ggg cgg gca   16198
Glu Lys Glu Ser Leu Gln Val Ile Thr Val Gly Glu Ala Gly Arg Ala
               4685                4690                4695
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caa gtg cgc gta ttg cac tgg gag atc ggc aag ccg gat gac ctc gat   16246
Gln Val Arg Val Leu His Trp Glu Ile Gly Lys Pro Asp Asp Leu Asp
          4700                4705                4710

gag gac tcg gtg cgt tac agt tac gat aac ctg gtg ggc agc agc cag   16294
Glu Asp Ser Val Arg Tyr Ser Tyr Asp Asn Leu Val Gly Ser Ser Gln
       4715                4720                4725

ctg gag ctg gac aga gag ggt tac ctt atc agt gag gag gag ttc tac   16342
Leu Glu Leu Asp Arg Glu Gly Tyr Leu Ile Ser Glu Glu Glu Phe Tyr
   4730                4735                4740

ccg tat ggc gga acg gct gtt ctg acg gcg cga agt gag gtt gag gct   16390
Pro Tyr Gly Gly Thr Ala Val Leu Thr Ala Arg Ser Glu Val Glu Ala
4745                4750                4755                4760

gac tac aaa act atc cga tac tca ggc aag gag cgt gac gcg acg ggg   16438
Asp Tyr Lys Thr Ile Arg Tyr Ser Gly Lys Glu Arg Asp Ala Thr Gly
               4765                4770                4775

ctg gat tat tac ggt tat cgg tat tac cag cca tgg gca ggg cgc tgg   16486
Leu Asp Tyr Tyr Gly Tyr Arg Tyr Tyr Gln Pro Trp Ala Gly Arg Trp
          4780                4785                4790

ctc tcc acg gac ccg gca ggc acg gtg gac ggg ctg aac ctg ttc cgc   16534
Leu Ser Thr Asp Pro Ala Gly Thr Val Asp Gly Leu Asn Leu Phe Arg
       4795                4800                4805

atg gtg cgg aat aat ccc gtc acg ctg ttt gac agc aac ggg cgg atc   16582
Met Val Arg Asn Asn Pro Val Thr Leu Phe Asp Ser Asn Gly Arg Ile
   4810                4815                4820

agt act ggt cag gag gcc aga cga tta gtg ggg gaa gca ttt gtt cat   16630
Ser Thr Gly Gln Glu Ala Arg Arg Leu Val Gly Glu Ala Phe Val His
4825                4830                4835                4840

ccg tta cac atg cct gtt ttt gaa aga att tct gta gag aga aag att   16678
Pro Leu His Met Pro Val Phe Glu Arg Ile Ser Val Glu Arg Lys Ile
               4845                4850                4855

tca atg agc gta agg gaa gct ggc att tat act att tca gcg ctg ggt   16726
Ser Met Ser Val Arg Glu Ala Gly Ile Tyr Thr Ile Ser Ala Leu Gly
          4860                4865                4870

gaa ggt gca gca gca aaa ggc cat aat att cta gag aaa acc att aaa   16774
Glu Gly Ala Ala Ala Lys Gly His Asn Ile Leu Glu Lys Thr Ile Lys
       4875                4880                4885

ccc ggt tcc ctg aag gct atc tat ggt gat aaa gct gag tca att ctt   16822
Pro Gly Ser Leu Lys Ala Ile Tyr Gly Asp Lys Ala Glu Ser Ile Leu
   4890                4895                4900

gga ctg gca aaa cgt agc ggt ctc gtt ggc cga gta gga cag tgg gat   16870
Gly Leu Ala Lys Arg Ser Gly Leu Val Gly Arg Val Gly Gln Trp Asp
4905                4910                4915                4920

gca tca ggt gta cgt gga att tat gcg cac aac aga ccg ggt ggt gag   16918
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Ala Ser Gly Val Arg Gly Ile Tyr Ala His Asn Arg Pro Gly Gly Glu
                4925                4930                4935

gat ttg gtt tat cct gtc agc ctg cag aat act tct gcc aat gaa att   16966
Asp Leu Val Tyr Pro Val Ser Leu Gln Asn Thr Ser Ala Asn Glu Ile
            4940                4945                4950

gtt aat gca tgg ata aaa ttt aaa atc atc acg ccc tac acc ggg gat   17014
Val Asn Ala Trp Ile Lys Phe Lys Ile Ile Thr Pro Tyr Thr Gly Asp
        4955                4960                4965

tat gac atg cac gat att att aaa ttc tct gat ggg aaa ggg cat gtg   17062
Tyr Asp Met His Asp Ile Ile Lys Phe Ser Asp Gly Lys Gly His Val
    4970                4975                4980

cct aca gcg gaa agt agt gag gaa aga gga gta aaa gat cta att aat   17110
Pro Thr Ala Glu Ser Ser Glu Glu Arg Gly Val Lys Asp Leu Ile Asn
4985                4990                4995                5000

aaa ggt gtt gcg gag gtc gat cct tcc aga ccc ttt gag tat aca gcg   17158
Lys Gly Val Ala Glu Val Asp Pro Ser Arg Pro Phe Glu Tyr Thr Ala
                5005                5010                5015

atg aat gtt att cgc cat gga cca cag gtg aac ttt gtt ccc tat atg   17206
Met Asn Val Ile Arg His Gly Pro Gln Val Asn Phe Val Pro Tyr Met
            5020                5025                5030

tgg gaa cat gag cac gat aaa gtc gtt aat gat aat ggt tat ctg ggg   17254
Trp Glu His Glu His Asp Lys Val Val Asn Asp Asn Gly Tyr Leu Gly
        5035                5040                5045

gtg gta gct agc ccg ggg ccg ttc ccg gta gcg atg gta cat cag ggg   17302
Val Val Ala Ser Pro Gly Pro Phe Pro Val Ala Met Val His Gln Gly
    5050                5055                5060

gaa tgg act gtt ttt gac aac agt gaa gaa ctg ttt aat ttc tat aaa   17350
Glu Trp Thr Val Phe Asp Asn Ser Glu Glu Leu Phe Asn Phe Tyr Lys
5065                5070                5075                5080

tct aca aat aca cct ctt cct gaa cac tgg tcc caa gat ttt atg gac   17398
Ser Thr Asn Thr Pro Leu Pro Glu His Trp Ser Gln Asp Phe Met Asp
                5085                5090                5095

aga ggg aaa gga ata gtc gca act cct cgg cat gct gaa ctt ctt gat   17446
Arg Gly Lys Gly Ile Val Ala Thr Pro Arg His Ala Glu Leu Leu Asp
            5100                5105                5110

aaa cga cga gtc atg tac taa tcgtaacgat ttcctgcctt acccaaagta      17497
Lys Arg Arg Val Met Tyr
        5115

tacagcccgg tgagacattt tctctgtctc atttgggttg tttttgtctc atctgcatgt 17557

tatgtcttcc ctcatctaaa gtctaacgag acatttttag caaaatggca ctttacggtt 17617

atgttcgcgt ttcaaccgac ggtccggatt ttactctgta aatacagaca cttcgcgcag 17677

cctgctgcga aattatccgt gcgaaaaaag ccagcggcag cagccgggat ggacgaaatg 17737
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aactgcagct tctgctggct tttttgcggc caggcaacat gctgatggtt acgtgagttg 17797

atcggctgcc accaaaaagt ccggagcgtg cggcccagat cgccgcaata atactgctgt 17857

atggtatttc catcaccact gtatatcgca cactctgggc cttccagaaa ccccataccg 17917

cacaccggtg tgatcgctgg aagccccggg cattaccgcc gtctgtactc gaacactatt 17977

gtggacttga tggttaggag attgaatcga ccatttttga gatccctaac catagatcgt 18037

agagttgcac actcccagat ggcgtggctt agcgagcgat tatgcttaaa aattcatgtt 18097

ttgctgtgtt tttaatccaa aacctgcttt tcaggcgcac ttatccagct acggggtctg 18157

aagccatcgt ttttttgccg tacgatgtag cctgtcagag agcatttttg tggcgtgctc 18217

gcccgctacg gtaccggcgg caaaacgcag ccggcctttg cagaggatgc actggtacgg 18277

atcggtgccc aggaagcctt tcatcagcac cgcgaacccg ggccgtttcg gtttctcccg 18337

taccgtcatc tccagcgcgt cgtaaacctt cggcagcagc gtgcccgttt gcggttggcc 18397

agaaaaccat agtaacgcac cattttaaaa tgccgtgcag ggatatggct gacgtaacgc 18457

tgcagcatct cctcctggct gattttctgg cgtttgtgct gctgcgtacg gtgatcgtaa 18517

tactgatgca ccacggcccc gccgcggtag tggcgtagct gagaagccgc caccggcggg 18577

cgcttcaggt accgggccag gtatttcacg ctgcgccagg cgccgcgggt ctttttggca 18637

aaattcactt tccaggggcg gcggtattgc gcatgcaggg tcttcgttgc ggatatggcc 18697

gagacccggc agggcgccag gattgatgcg cagcaggtga acgacggcat tgcgccagat 18757

ggcttccacc tctttctttt taaagaacag ctgccgccag acgtggtgtt tgacgtcaag 18817

accgccgcgg gtaacggaga cgtggatatg cggatgttga ttgagctgcc ggccttaggt 18877

gtggagcgcg caaaaaatgc cggcctcgat gccctgccgg cgtgcccagc ggagcatggc 18937

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 144 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (ORF 1)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Ile Ser Ser Arg Gly Ile Ala Leu Ile Lys Glu Phe Glu Gly
1 5 10 15

Leu Arg Leu His Ala Tyr Arg Cys Ala Ala Asp Val Trp Thr Val Gly
20 25 30

Tyr Gly His Thr Ala Gly Val Thr Lys Gly Asp Ile Ile Thr Val Asp
35 40 45

Glu Ala Gln Thr Met Leu Thr Asn Asp Ile Thr Val Phe Glu Arg Ala
50 55 60

Val Ser Gln Ala Val Ala Val Pro Leu Asn Gln Ser Gln Tyr Asp Ala
65 70 75 80

Leu Val Ser Leu Val Phe Asn Ile Gly Gln Gly Asn Phe Lys Arg Ser
85 90 95

Thr Leu Leu Lys Lys Leu Asn Lys Gln Asp Tyr Val Gly Ala Gly Asn
100 105 110

Glu Phe Leu Arg Trp Thr Arg Ala Asn Gly Lys Val Leu Pro Gly Leu
115 120 125

Ile Arg Arg Arg Glu Ala Glu Arg Val Leu Phe Glu Lys Leu Gly
130 135 140

Ala

(2) INFORMATION FOR SEQ ID NO: 3:

(i). SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (ORF 2)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Ser Pro Ser Pro Leu Thr Gly Ala Ala Leu Met Glu Thr Lys Met
1 5 10 15

Lys Ile His Tyr Gln Val Ala Ala Val Val Leu Thr Gly Val Met Val
20 25 30

Trp Gly Leu Ser His Trp Arg Tyr Thr Val Gly Tyr His Ala Ala Asp
35 40 45

Thr Gln Trp Gln Gln Arg Gln Ala Glu Gln Glu Arg Ala Asp Ala Leu
50 55 60

Ala Leu Leu Ala Ala Glu Thr Arg Glu Arg Lys Trp Glu Gln Gln Arg
65 70 75 80

Gln Thr Asp Met Asn Lys Val Ala Ile His Ala Glu Glu Glu Leu Ala
85 90 95

Ala Ala Arg Asp Ala Ala Ala Asp Ala Gln Arg Thr Gly Gln Arg Leu
100 105 110

Gln His Thr Val Thr Thr Leu Gln Arg Gln Leu Ala Ser Arg Glu Thr
115 120 125

Arg Arg Leu Ser Ala Ala Thr Ala Ile Gly Thr Asp Asp Leu Gly Gly
130 135 140

Gln Pro Gly Val Leu Phe Ala Glu Leu Phe Arg Arg Ala Asp Gln Arg
145 150 155 160

Ala Gly Glu Leu Ala Ala Tyr Ala Asp Arg Thr Arg Val Lys Trp Gln
165 170 175

Ala Cys Gly Arg Ala Tyr Gln Ala Ala Thr His Glu Ala Glu Lys
180 185 190

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2376 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (SepA)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Met Arg Gln Asp Ile Met Tyr Asn Ile Asp Asp Ile Leu Glu Lys Val
 1               5                  10                  15

Asn Ala Pro Arg Ala Arg Leu Ser Glu Glu Asn Asp Thr Ala Val Thr
            20                  25                  30

Leu Thr Asp Leu Phe Ser Arg Ser Phe Pro Glu Val Lys Lys Ile Thr
        35                  40                  45

Gly Asp Ser Leu Ser Trp Gly Glu Val Cys Tyr Leu Tyr Ser Gln Ala
    50                  55                  60

Gln His Glu Gln Lys Glu Asn Arg Leu Thr Glu Ser Arg Ile Leu Ala
65                  70                  75                  80

Arg Ala Asn Pro Leu Leu Val Asn Ala Val Arg Leu Gly Ile Arg Gln
                85                  90                  95

Ala Ala Gly Ser Arg Ser Tyr Asp Asp Trp Phe Gly Ser Arg Ala Asp
            100                 105                 110

Arg Phe Ala Arg Pro Gly Ser Val Ala Ser Met Phe Ser Pro Ala Ala
        115                 120                 125

Tyr Leu Thr Glu Leu Tyr Arg Glu Ala Lys Asp Leu His Pro Asp Thr
    130                 135                 140

Ser Leu Phe Arg Leu Asp Ile Arg Arg Pro Asp Leu Ala Ala Leu Ala
145                 150                 155                 160

Leu Ser Gln Asn Asn Met Asp Asp Glu Leu Ser Thr Leu Ser Leu Ser
                165                 170                 175

Asn Glu Leu Leu Tyr Arg Gly Ile Gly Ala Ala Glu Gly Leu Asp Asp
            180                 185                 190
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Asp Ser Val Arg Glu Leu Leu Ala Gly Tyr Arg Leu Thr Gly Leu Thr
195 200 205

Pro Tyr His Trp Ala Tyr Glu Ala Ala Arg Gln Ala Ile Leu Val Gln
210 215 220

Asp Pro Thr Leu Met Gly Phe Ser Arg Asn Pro Asp Val Ala Gln Leu
225 230 235 240

Met Asp Pro Ala Ser Met Leu Ala Ile Glu Ala Asp Ile Ser Pro Glu
245 250 255

Leu Tyr Gln Ile Leu Ala Glu Glu Ile Thr Thr Asp Ser Tyr Glu Ala
260 265 270

Leu Trp Ser Lys Asn Phe Gly Asp Met Pro Pro Ser Ser Leu Leu Ser
275 280 285

Tyr Asp Ala Leu Ala Thr Phe Tyr Asp Leu Asp Tyr Asp Glu Leu Thr
290 295 300

Ser Leu Leu Ser Leu Arg Leu Asp Phe Ser Asn Pro Asn Asn Glu Tyr
305 310 315 320

Tyr Ile Asn Ser Gln Leu Ser Val Val Thr Leu Asn Glu Ser Thr Gly
325 330 335

Leu Ile Thr Ile His His Tyr Leu Arg Thr Leu Gly Gly Asp Ser Gln
340 345 350

Gln Ile Asn Pro Glu Leu Ile Pro Tyr Gly Asp Gly Thr Tyr Leu Tyr
355 360 365

Asn Phe Ser Val Val Ser Thr Ile Ser Glu Asp Ser Phe Lys Leu Gly
370 375 380

Ser Leu Gly Ser Asn Ser Ser Asn Leu Tyr Ser Gly Asp Tyr Gln Leu
385 390 395 400

Gln Lys Gly Val Arg Tyr Ser Ile Pro Val Glu Ile Asp Glu Gly Lys
405 410 415

Leu Asn Asp Gly Ile Thr Ile Gly Leu Ser Arg Lys Gly Gly Gly Tyr
420 425 430

Tyr Ser Thr Val Asn Phe Thr Leu Ile Glu Tyr Asp Pro Ala Ile Phe
435 440 445

Ile Leu Lys Leu Asn Lys Val Ile Arg Leu Tyr Lys Ala Thr Gly Met
450 455 460

Thr Thr Ala Glu Ile Tyr Gln Ile Thr Asn Ile Leu Asn Asn Gly Leu
465 470 475 480

Thr Ile Asp His Ala Val Leu Ser Lys Ile Phe Leu Val Arg Tyr Leu
485 490 495

Met Arg His Tyr Gln Leu Asp Val Ala Arg Ser Leu Ile Leu Cys Asn

500 505 510

Gly Thr Ile Ser Asp Gln Ala Phe Ser Gly Glu Thr Gly Leu Phe Thr
515 520 525

Thr Leu Phe Asn Thr Pro Pro Leu Asn Gly Gln Leu Phe Ser Ala Asp
530 535 540

Asp Thr Pro Leu Asp Leu Arg Ser Glu Ala Pro Glu Asp Ala Phe Arg
545 550 555 560

Leu Ser Val Leu Lys Arg Ala Phe Asn Ile Ser Ala Ser Gly Leu Ser
565 570 575

Thr Leu Trp Gln Leu Ala Ser Gly Asp Ser Ser Ala Gly Phe Ser Cys
580 585 590

Ser Ala Asp Asn Ile Ala Ala Leu Tyr Arg Val Lys Leu Leu Ala Asp
595 600 605

Ile His Asp Leu Ser Ala Gly Glu Leu Ser Met Leu Leu Ser Val Ser
610 615 620

Pro Phe Ser Gly Val Ala Ala Gly Ser Leu Ser Asp Asn Glu Leu Thr
625 630 635 640

Gln Phe Leu Tyr Gln Thr Thr Thr Trp Leu Thr Glu Gln Gly Trp Thr
645 650 655

Val Ser Asp Val Phe Leu Met Leu Thr Thr Gln Tyr Gly Thr Leu Leu
660 665 670

Thr Pro Asp Ile Glu Asn Leu Leu Ala Ser Leu Arg Asn Gly Leu Ser
675 680 685

Gly Arg Glu Leu Phe Pro Glu Thr Leu Pro Gly Asp Gly Ala Pro Phe
690 695 700

Ile Ala Ala Ala Met Gln Leu Asp Ala Thr Asp Thr Ala Lys Ala Met
705 710 715 720

Leu Thr Trp Ala Asp Gln Leu Lys Pro Glu Gly Leu Thr Leu Thr Glu
725 730 735

Phe Ile Leu Leu Val Met Asn Ala Ala Pro Asn Asp Glu Gln Ala Gly
740 745 750

Gln Met Ala Gly Phe Cys Gln Ala Leu Trp Gln Leu Ala Leu Ile Ile
755 760 765

Arg Ser Thr Gly Leu Ser Thr Arg Glu Leu Thr Leu Leu Val Ser Gln
770 775 780

Pro Gly Arg Phe Arg Thr Gly Trp His His Leu Pro His Asp Leu Pro
785 790 795 800

Ala Leu Arg Asp Ile Thr Arg Phe His Ala Val Val Asn Arg Ser Gly
805 810 815

Ser His Ala Gly Glu Val Leu Thr Ala Leu Glu Thr Gly Glu Leu Ser
820 825 830

Ser Ala Leu Leu Ala Arg Ala Leu Ser Gln Asn Glu Gln Asp Val Thr
835 840 845

Gly Ala Leu Ala Gln Val Arg Gly Ala Gly Glu Gln Asp Asn Ser Val
850 855 860

Phe Thr Ser Trp Glu Glu Val Asp Gln Ala Glu Gln Trp Leu Asp Met
865 870 875 880

Ser Glu Thr Leu Ser Ile Thr Pro Ser Gly Leu Ala Ser Leu Ile Ala
885 890 895

Leu Lys Tyr Ile Asn Val Ser Asp Asp Ser Ala Pro Leu Tyr Ser Gln
900 905 910

Trp Gln Val Val Ser Gly Leu Leu Gln Ala Gly Leu Lys Ser Ser Gln
915 920 925

Ser Ser Ala Leu His Asp Tyr Leu Glu Glu Gly Thr Ser Ser Ala Leu
930 935 940

Cys Ala Tyr Tyr Leu Arg Asn Leu Ala Pro Asn Met Val Ser Gly Arg
945 950 955 960

Asp Asp Leu Phe Gly Tyr Leu Leu Leu Asp Asn Gln Val Ser Ala Lys
965 970 975

Val Lys Thr Thr Arg Ile Ala Glu Ala Ile Ala Gly Ile Arg Leu Tyr
980 985 990

Ile Asn Arg Ala Leu Asn Gly Ile Glu Leu Ser Ala Met Ala Glu Val
995 1000 1005

Arg Gly Arg Gln Phe Phe Thr Asp Trp Asp Thr Phe Asn Lys Arg Tyr
1010 1015 1020

Ser Thr Trp Ala Gly Val Ser Glu Leu Val Tyr Tyr Pro Glu Asn Tyr
025 1030 1035 1040

Leu Asp Pro Thr Val Arg Ile Gly Gln Thr Gly Met Met Asp Thr Leu
1045 1050 1055

Leu Gln Ser Val Ser Gln Ser Ser Ile Asn Arg Asp Thr Val Glu Asp
1060 1065 1070

Ala Phe Lys Thr Tyr Leu Thr Thr Phe Glu Gln Ile Ala Asn Leu Asn
1075 1080 1085

Thr Val Ser Gly Tyr His Asp Asn Ala Ser Met Thr Gln Gly Thr Thr
1090 1095 1100

Trp Tyr Val Gly Arg Ser Ile Thr Asp Gln Thr Asn Trp Tyr Trp Arg
105 1110 1115 1120

Ser Ala Asn His Ser Lys Ile Gln Asp Ser Met Met Pro Ala Asn Ala
1125 1130 1135

Trp Thr Gly Trp Thr Lys Ile Asn Cys Gly Met Asn Pro Trp Ser Asp
1140 1145 1150

Leu Val Cys Ser Val Phe Phe Asn Ser Arg Leu Tyr Val Val Trp Val
1155 1160 1165

Glu Glu Asn Gln Ser Ala Asp Thr Glu Ala Glu Ser Thr Thr Thr Thr
1170 1175 1180

Gln Gln Ser Tyr Thr Leu Lys Leu Ser Phe Arg Arg Tyr Asp Gly Thr
185 1190 1195 1200

Trp Ser Ser Pro Val Ser Phe Asp Ile Thr Gly Asn Ile Ala Phe Pro
1205 1210 1215

Glu Thr Gln Gly Met His Val Thr Cys Asn Pro Leu Thr Glu Gln Leu
1220 1225 1230

Tyr Cys Ala Phe Tyr Ser Val Thr Ser Lys Pro Asp Phe Asp Asn Ala
1235 1240 1245

Gln Leu Ile Ser Val Asp Asn Asp Met Thr Leu Asn Val Ile Ser Asp
1250 1255 1260

Ile Gly Ile Phe Lys Ser Val Ser His Glu Phe Asn Thr Ser Thr Glu
265 1270 1275 1280

Lys Phe Ile Asn Asn Val Phe Ser Asp Pro Ser Ala Asn Tyr Phe Val
1285 1290 1295

Ser Ala Thr Ser Leu Ile Asp Asp Val Ile His Ser Asp Phe Ser Leu
1300 1305 1310

Leu Asn Ser Lys Thr Thr Ser Thr Val Phe Thr Asn Glu Asp Ser Ser
1315 1320 1325

Leu Leu Thr Pro Glu Leu His Ile Thr Ala Asn Val Ser Cys Phe Val
1330 1335 1340

Ser Thr Ala Gly Ile Ala Thr Gln Ser Thr Ile Glu Lys Phe Val Gln
345 1350 1355 1360

Ala Gly Ile Glu Phe Glu Glu Ile Asn Phe Tyr Ala Gly Gln Ala Ala
1365 1370 1375

Gly Gly Phe Asp Gly Phe Val Gly Val Asp Val Ser Asn Ser Lys Val
1380 1385 1390

Tyr Gln Val Gly Lys Glu Ala Val Gly Val Thr Val Lys Ser Tyr Ser
1395 1400 1405

Val Thr Gly Val Ser Gly Ser Val Glu Leu Phe Ile Asp Ser Ser Asn
1410 1415 1420

Lys Tyr Phe Ser Gly Ile Leu Ser Asp Lys Met Ile Thr Ala Leu Ile

425 1430 I435 1440

Ser Gly Ser Thr Ser Lys Val Asn Tyr Val Ser Ser Ile Gly Ser Gln
1445 1450 1455

Asp Phe Trp Ser Val Lys Ser Leu Met Pro Ala Leu Gln Ile Tyr Glu
1460 1465 1470

Leu Ile Asp Asp Ile Ile Leu Thr Ser Gly Val Asn Gly Thr Glu Ile
1475 1480 1485

Lys Ser Trp Pro Ser Ala Glu Trp Tyr Asn Asp Lys Leu Ser Leu Gln
1490 1495 1500

Ser Gly Asn Asn Leu Phe Asn Thr Lys Ser Leu Ser Phe Thr Val Asn
505 1510 1515 1520

Thr Ser Asp Ile Val Glu Asp Glu Phe Asp Val Thr Phe Thr Phe Thr
1525 1530 1535

Ala Val Asp Gln Asn Asn Val Val Leu Ala Ala Arg Thr Ala Ile Leu
1540 1545 1550

Thr Val Ile Arg Asn Ile Asn Asn Asp Thr Ser Val Ile Ala Leu Arg
1555 1560 1565

Lys Asn Thr Arg Gly Ala Gln Tyr Ile Arg Phe Thr Ala Gly Asn Asp
1570 1575 1580

Val Ala Leu Ile Arg Leu Asn Thr Leu Phe Ala Arg Gln Leu Val Asp
585 1590 1595 1600

Arg Ala Asn Thr Gly Ile Asp Thr Ile Leu Ser Met Glu Thr Gln Arg
1605 1610 1615

Leu Thr Glu Pro Ala Leu Glu Glu Gly Ser Asp Val Phe Met Asp Phe
1620 1625 1630

Ser Gly Ala Asn Ala Leu Tyr Phe Trp Glu Leu Phe Tyr Tyr Thr Pro
1635 1640 1645

Met Met Val Phe Gln Arg Leu Leu Gln Glu Gln His Phe Pro Glu Ala
1650 1655 1660

Thr Arg Trp Leu Gln Tyr Val Trp Asn Pro Ala Gly His Val Val Asn
665 1670 1675 1680

Gly Val Leu Gln Asn Tyr Thr Trp Asn Val Arg Pro Leu Glu Glu Asp
1685 1690 1695

Thr Gly Trp Asn Asp Ser Pro Leu Asp Ser Ile Asp Pro Asp Ala Ile
1700 1705 1710

Ala Gln Tyr Asp Pro Met His Tyr Lys Val Ala Thr Phe Met Ser Tyr
1715 1720 1725

Leu Asp Leu Leu Ile Ala Arg Gly Asp Ala Ala Tyr Arg Leu Leu Glu
1730 1735 1740

```
Arg Asp Thr Leu Asn Glu Ala Arg Met Trp Tyr Val Gln Ala Leu Asn
745                 1750                1755                1760

Leu Leu Gly Asp Glu Pro Tyr Ile Ser Phe Asp Ala Asp Trp Ser Ala
                1765                1770                1775

Leu Thr Leu Gly Asp Ala Ala Ser Glu Val Thr Arg Arg Asp Tyr Gln
            1780                1785                1790

Glu Ala Leu Leu Ala Val Arg Arg Leu Val Pro Ala Pro Glu Thr Arg
        1795                1800                1805

Thr Ala Asn Ser Leu Thr Ala Leu Phe Leu Pro Gln Gln Asn Glu Val
    1810                1815                1820

Leu Lys Gly Tyr Trp Gln Thr Leu Ala Gln Arg Leu His Asn Leu Arg
825                 1830                1835                1840

His Asn Leu Ser Ile Asp Gly Gln Pro Leu Ser Leu Ser Val Tyr Ala
                1845                1850                1855

Thr Pro Ser Glu Pro Ser Ala Leu Gln Ser Ala Val Val Asn Ser Ala
            1860                1865                1870

Gln Gly Ala Ala Ala Leu Pro Ala Ala Val Met Pro Leu Tyr Ser Phe
        1875                1880                1885

Pro Val Met Leu Glu Asn Ala Arg Gly Met Val Ser Leu Leu Thr Gly
    1890                1895                1900

Phe Gly Asn Thr Leu Leu Gly Ile Thr Glu Arg Gln Asp Ala Glu Ala
905                 1910                1915                1920

Leu Ala Lys Leu Leu Gln Thr Gln Gly Ser Glu Leu Ile Arg Gln Gly
                1925                1930                1935

Leu Arg Gln Gln Asp Asn Val Leu Glu Glu Ile Asp Ala Asp Ile Ala
            1940                1945                1950

Ala Leu Glu Glu Ser Arg Arg Gly Ala Gln Met Arg Phe Glu Arg Tyr
        1955                1960                1965

Lys Val Leu Tyr Glu Ala Asp Val Asn Thr Gly Glu Lys Gln Ala Met
    1970                1975                1980

Asp Leu Tyr Leu Ser Ser Ser Val Leu Ser Ala Ser Thr Ala Ala Leu
985                 1990                1995                2000

Phe Leu Ala Glu Ala Ala Ala Asp Met Leu Pro Asn Ile Tyr Gly Leu
                2005                2010                2015

Ala Val Gly Gly Ser Arg Tyr Gly Ala Leu Phe Lys Ala Thr Ala Ile
            2020                2025                2030

Gly Ile Gln Val Ser Ser Asp Ala Thr Arg Ile Ser Ala Asp Lys Ile
        2035                2040                2045
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Ser Gln Ser Glu Val Tyr Arg Arg Arg Arg Glu Glu Trp Glu Ile Gln
2050 2055 2060

Arg Asp Ser Ala Gln Ser Asp Val Ala Gln Ile Asp Ala Gln Leu Ala
065 2070 2075 2080

Ala Met Ala Val Arg Arg Glu Gly Ala Glu Leu Gln Lys Thr Tyr Leu
2085 2090 2095

Glu Thr Gln Gln Thr Gln Ala Gln Ala Gln Leu Ala Phe Leu Gln Ser
2100 2105 2110

Lys Phe Asn Asn Thr Ala Leu Tyr Ser Trp Leu Arg Gly Arg Leu Ser
2115 2120 2125

Ala Ile Tyr Tyr Gln Phe Tyr Asp Leu Ala Val Ser Arg Cys Leu Met
2130 2135 2140

Ala Gln Gln Ala Trp Gln Trp Asp Lys Phe Glu Thr Arg Ser Phe Ile
145 2150 2155 2160

Gln Pro Gly Ala Trp Met Gly Ala Asn Ala Gly Leu Leu Ala Gly Glu
2165 2170 2175

Thr Leu Met Leu Asn Leu Ala Gln Met Glu Gln Ala Trp Leu Thr Gly
2180 2185 2190

Asp Glu Arg Ala Ile Glu Val Thr Arg Thr Val Cys Leu Ser Glu Val
2195 2200 2205

Tyr Thr Ser Leu Ala Glu Asp Ala Ala Phe Ser Leu Ala Asp Lys Val
2210 2215 2220

Val Glu Leu Val Ser Asn Gly Ser Gly Ser Ala Gly Thr Lys Ser Asn
225 2230 2235 2240

Gly Leu Gln Met Asp Gln Gln Gln Leu Glu Ala Thr Leu Lys Leu Ala
2245 2250 2255

Asp Leu Gly Ile Gly Asn Asp Tyr Pro Val Ser Leu Gly Thr Met Arg
2260 2265 2270

Arg Ile Lys Gln Ile Ser Val Thr Leu Pro Ala Leu Val Gly Pro Tyr
2275 2280 2285

Gln Asp Val Arg Ala Val Leu Ser Tyr Gly Gly Ser Met Val Met Pro
2290 2295 2300

Arg Gly Cys Ser Ala Leu Ala Val Ser His Gly Met Asn Asp Ser Gly
305 2310 2315 2320

Gln Phe Gln Leu Asp Phe Asn Asp Pro Arg Tyr Leu Pro Phe Glu Gly
2325 2330 2335

Leu Pro Val Asp Asp Thr Gly Thr Leu Thr Leu Ser Phe Pro Asp Ala
2340 2345 2350

Asp Gly Lys Gln Gln Ala Met Leu Leu Ser Leu Ser Asp Ile Ile Leu
2355 2360 2365

His Ile Arg Tyr Thr Ile Ile Ser
2370 2375

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1429 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (SepB)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Gln Asn His Gln Asp Met Ala Ile Thr Ala Pro Thr Leu Pro Ser
 1               5                  10                  15

Gly Gly Gly Ala Val Thr Gly Leu Lys Gly Asp Ile Ala Ala Ala Gly
            20                  25                  30

Pro Asp Gly Ala Ala Thr Leu Ser Ile Pro Leu Pro Val Ser Pro Gly
        35                  40                  45

Arg Gly Tyr Ala Pro Thr Gly Ala Leu Asn Tyr His Ser Arg Ser Gly
    50                  55                  60
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Asn Gly Pro Phe Gly Ile Gly Trp Gly Ile Gly Gly Ala Ala Val Gln
65 70 75 80

Arg Arg Thr Arg Asn Gly Ala Pro Thr Tyr Asp Asp Thr Asp Glu Phe
85 90 95

Thr Gly Pro Asp Gly Glu Val Leu Val Pro Ala Leu Thr Ala Ala Gly
100 105 110

Thr Gln Glu Ala Arg Gln Ala Thr Ser Leu Leu Gly Ile Asn Pro Gly
115 120 125

Gly Ser Phe Asn Val Gln Val Tyr Arg Ser Arg Thr Glu Gly Ser Leu
130 135 140

Ser Arg Leu Glu Arg Trp Leu Pro Ala Asp Glu Thr Glu Thr Glu Phe
145 150 155 160

Trp Val Leu Tyr Thr Pro Asp Gly Gln Val Ala Leu Leu Gly Arg Asn
165 170 175

Ala Gln Ala Arg Ile Ser Asn Pro Thr Ala Pro Thr Gln Thr Ala Val
180 185 190

Trp Leu Met Glu Ser Ser Val Ser Leu Thr Gly Glu Gln Met Tyr Tyr
195 200 205

Gln Tyr Arg Ala Glu Asp Asp Asp Gly Cys Asp Glu Ala Glu Arg Asp
210 215 220

Ala His Pro Gln Ala Gly Ala Gln Arg Tyr Pro Val Ala Val Trp Tyr
225 230 235 240

Gly Asn Arg Gln Ala Ala Arg Thr Leu Pro Ala Leu Val Ser Thr Pro
245 250 255

Ser Met Asp Ser Trp Leu Phe Ile Leu Val Phe Asp Tyr Gly Glu Arg
260 265 270

Ser Ser Val Leu Ser Glu Ala Pro Ala Trp Gln Thr Pro Gly Ser Gly
275 280 285

Glu Trp Leu Cys Arg Gln Asp Cys Phe Ser Gly Tyr Glu Phe Gly Phe
290 295 300

Asn Leu Arg Thr Arg Arg Leu Cys Arg Gln Val Leu Met Phe His Tyr
305 310 315 320

Leu Gly Val Leu Ala Gly Ser Ser Gly Ala Asn Asp Ala Pro Ala Leu
325 330 335

Ile Ser Arg Leu Leu Leu Asp Tyr Arg Glu Ser Pro Ser Leu Ser Leu
340 345 350

Leu Glu Asn Val His Gln Val Ala Tyr Glu Ser Asp Gly Thr Ser Cys
355 360 365

Ala Leu Pro Ala Leu Ala Leu Gly Trp Gln Thr Phe Thr Pro Pro Thr
370 375 380

Leu Ser Ala Trp Gln Thr Arg Asp Asp Met Gly Lys Leu Ser Leu Leu
385 390 395 400

Gln Pro Tyr Gln Leu Val Asp Leu Asn Gly Glu Gly Val Val Gly Ile
405 410 415

Leu Tyr Gln Asp Ser Gly Ala Trp Trp Tyr Arg Glu Pro Val Arg Gln
420 425 430

Ser Gly Asp Asp Pro Asp Ala Val Thr Trp Gly Ala Ala Ala Ala Leu
435 440 445

Pro Thr Met Pro Ala Leu His Asn Ser Gly Ile Leu Ala Asp Leu Asn
450 455 460

Gly Asp Gly Arg Leu Glu Trp Val Val Thr Ala Pro Gly Val Ala Gly
465 470 475 480

Met Tyr Asp Arg Thr Pro Gly Arg Asp Trp Leu His Phe Thr Pro Leu
485 490 495

Ser Ala Leu Pro Val Glu Tyr Ala His Pro Lys Ala Val Leu Ala Asp
500 505 510

Ile Leu Gly Ala Gly Leu Thr Asp Met Val Leu Ile Gly Pro Arg Ser
515 520 525

Val Arg Leu Tyr Ser Gly Lys Asn Asp Gly Trp Asn Lys Gly Glu Thr
530 535 540

Val Gln Gln Thr Glu Arg Leu Thr Leu Pro Val Pro Gly Val Asp Pro
545 550 555 560

Arg Thr Leu Val Ala Phe Ser Asp Met Ala Gly Ser Gly Gln Gln His
565 570 575

Leu Thr Glu Val Arg Ala Asn Gly Val Arg Tyr Trp Pro Asn Leu Gly
580 585 590

His Gly Arg Phe Gly Gln Pro Val Asn Ile Pro Gly Phe Ser Gln Ser
595 600 605

Val Thr Thr Phe Asn Pro Asp Gln Ile Leu Leu Ala Asp Thr Asp Gly
610 615 620

Ser Gly Thr Thr Asp Leu Ile Tyr Ala Met Ser Asp Arg Leu Val Ile
625 630 635 640

Tyr Phe Asn Gln Ser Gly Asn Tyr Phe Ala Glu Pro His Thr Leu Leu
645 650 655

Leu Pro Lys Gly Val Arg Tyr Asp Arg Thr Cys Ser Leu Gln Val Ala
660 665 670

Asp Ile Gln Gly Leu Gly Val Pro Ser Leu Leu Leu Thr Val Pro His

675 680 685

Val Ala Pro His His Trp Val Cys His Leu Ser Ala Asp Lys Pro Trp
690 695 700

Leu Leu Asn Gly Met Asn Asn Asn Met Gly Ala Arg His Ala Leu His
705 710 715 720

Tyr Arg Ser Ser Val Gln Phe Trp Leu Asp Glu Lys Ala Glu Ala Leu
725 730 735

Ala Ala Gly Ser Ser Pro Ala Cys Tyr Leu Pro Phe Thr Leu His Thr
740 745 750

Leu Trp Arg Ser Val Val Gln Asp Glu Ile Thr Gly Asn Arg Leu Val
755 760 765

Ser Asp Val Leu Tyr Arg His Gly Val Trp Asp Gly Gln Glu Arg Glu
770 775 780

Phe Arg Gly Phe Gly Phe Val Glu Ile Arg Asp Thr Asp Thr Leu Ala
785 790 795 800

Ser Gln Gly Thr Ala Thr Glu Leu Ser Met Pro Ser Val Ser Arg Asn
805 810 815

Trp Tyr Ala Thr Gly Val Pro Ala Val Asp Glu Arg Leu Pro Glu Thr
820 825 830

Tyr Trp Gln Asn Asp Ala Ala Ala Phe Ala Asp Phe Ala Thr Arg Phe
835 840 845

Thr Val Gly Ser Gly Glu Asp Glu Gln Thr Tyr Thr Pro Asp Asp Ser
850 855 860

Lys Thr Phe Trp Leu Gln Arg Ala Leu Lys Gly Ile Leu Leu Arg Ser
865 870 875 880

Glu Leu Tyr Gly Ala Asp Gly Ser Ser Gln Ala Asp Ile Pro Tyr Ser
885 890 895

Val Thr Glu Ser Arg Pro Gln Val Arg Leu Val Glu Ala Asn Gly Asp
900 905 910

Tyr Pro Val Val Trp Pro Met Gly Ala Glu Ser Arg Thr Ser Val Tyr
915 920 925

Glu Arg Tyr His Asn Asp Pro Gln Cys Gln Gln Gln Ala Val Leu Leu
930 935 940

Ser Asp Glu Tyr Gly Phe Pro Leu Arg Gln Val Ser Val Asn Tyr Pro
945 950 955 960

Arg Arg Pro Pro Ser Ala Asp Asn Pro Tyr Pro Ala Ser Leu Pro Ala
965 970 975

Thr Leu Phe Ala Asn Ser Tyr Asp Glu Gln Gln Gln Ile Leu Arg Leu
980 985 990

Gly Leu Gln Gln Ser Ser Ala His His Leu Val Ser Leu Ser Glu Gly
995 1000 1005

His Trp Leu Leu Gly Leu Ala Glu Ala Ser Arg Asp Asp Val Phe Thr
1010 1015 1020

Tyr Ser Ala Asp Asn Val Pro Glu Gly Gly Leu Thr Leu Glu His Leu
025 1030 1035 1040

Leu Ala Pro Glu Ser Leu Val Ser Asp Ser Gln Val Gly Thr Leu Ala
1045 1050 1055

Gly Gln Gln Gln Val Trp Tyr Leu Asp Ser Gln Asp Val Ala Thr Val
1060 1065 1070

Ala Ala Pro Pro Leu Pro Pro Lys Val Ala Phe Ile Glu Thr Ala Val
1075 1080 1085

Leu Asp Glu Gly Met Val Ser Ser Leu Ala Ala Tyr Ile Val Asp Glu
1090 1095 1100

His Leu Glu Gln Ala Gly Tyr Arg Gln Ser Gly Tyr Leu Phe Pro Arg
105 1110 1115 1120

Gly Arg Glu Ala Glu Gln Ala Leu Trp Thr Gln Cys Gln Gly Tyr Val
1125 1130 1135

Thr Tyr Ala Gly Ala Glu His Phe Trp Leu Pro Leu Ser Phe Arg Asp
1140 1145 1150

Ser Met Leu Thr Gly Pro Val Thr Val Thr Arg Asp Ala Tyr Asp Cys
1155 1160 1165

Val Ile Thr Gln Trp Gln Asp Ala Ala Gly Ile Val Thr Thr Ala Asp
1170 1175 1180

Tyr Asp Trp Arg Phe Leu Thr Pro Val Arg Val Thr Asp Pro Asn Asp
185 1190 1195 1200

Asn Leu Gln Ser Val Thr Leu Asp Ala Leu Gly Arg Val Thr Thr Leu
1205 1210 1215

Arg Phe Trp Gly Thr Glu Asn Gly Ile Ala Thr Gly Tyr Ser Asp Ala
1220 1225 1230

Thr Leu Ser Val Pro Asp Gly Ala Ala Ala Ala Leu Ala Leu Thr Ala
1235 1240 1245

Pro Leu Pro Val Ala Gln Cys Leu Val Tyr Val Thr Asp Ser Trp Gly
1250 1255 1260

Asp Asp Asp Asn Glu Lys Met Pro Pro His Val Val Val Leu Ala Thr
265 1270 1275 1280

Asp Arg Tyr Asp Ser Asp Thr Gly Gln Gln Val Arg Gln Gln Val Thr
1285 1290 1295

Phe Ser Asp Gly Phe Gly Arg Glu Leu Gln Ser Ala Thr Arg Gln Ala
1300 1305 1310

Glu Gly Asn Ala Trp Gln Arg Gly Arg Asp Gly Lys Leu Val Thr Ala
1315 1320 1325

Ser Asp Gly Leu Pro Val Thr Val Ala Thr Asn Phe Arg Trp Ala Val
1330 1335 1340

Thr Gly Arg Ala Glu Tyr Asp Asn Lys Gly Leu Pro Val Arg Val Tyr
345 1350 1355 1360

Gln Pro Tyr Phe Leu Asp Ser Trp Gln Tyr Val Ser Asp Asp Ser Ala
1365 1370 1375

Arg Gln Asp Leu Tyr Ala Asp Thr His Phe Tyr Asp Pro Thr Ala Arg
1380 1385 1390

Glu Trp Gln Val Ile Thr Ala Lys Gly Glu Arg Arg Gln Val Leu Tyr
1395 1400 1405

Thr Pro Trp Phe Val Val Ser Glu Asp Glu Asn Asp Thr Val Gly Leu
1410 1415 1420

Asn Asp Ala Ser
425

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 973 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: PROTEIN (SepC)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Ser Thr Ser Leu Phe Ser Ser Thr Pro Ser Val Ala Val Leu Asp
 1               5                  10                  15

Asn Arg Gly Leu Leu Val Arg Glu Leu Gln Tyr Tyr Arg His Pro Asp
            20                  25                  30

Thr Pro Glu Glu Thr Asp Glu Arg Ile Thr Cys His Gln His Asp Glu
        35                  40                  45

Arg Gly Ser Leu Ser Gln Ser Ala Asp Pro Arg Leu His Ala Ala Gly
    50                  55                  60

Leu Thr Asn Phe Thr Tyr Leu Asn Ser Leu Thr Gly Thr Val Leu Gln
65                  70                  75                  80

Ser Val Ser Ala Asp Ala Gly Thr Ser Leu Glu Leu Ser Asp Ala Ala
                85                  90                  95

Gly Arg Ala Phe Leu Ala Val Thr Gly Ala Gly Thr Glu Asp Ala Val
            100                 105                 110

Thr Arg Thr Trp Gln Tyr Glu Asp Asp Thr Leu Pro Gly Arg Pro Leu
        115                 120                 125

Ser Ile Thr Glu Gln Val Thr Gly Glu Ala Ala Gln Ile Thr Glu Arg
    130                 135                 140

Phe Val Tyr Ala Gly Asn Thr Asp Ala Glu Lys Ile Leu Asn Leu Ala
145                 150                 155                 160

Gly Gln Cys Val Ser His Tyr Asp Thr Ala Gly Leu Val Gln Thr Asp
                165                 170                 175

Ser Ile Ala Leu Ser Gly Val Pro Leu Ala Val Thr Arg Gln Leu Leu
            180                 185                 190

Pro Asp Ala Ala Gly Ala Asn Trp Met Gly Glu Asp Ala Ser Ala Trp
        195                 200                 205

Asn Asp Leu Leu Asp Gly Glu Thr Phe Phe Thr Gln Thr His Ala Asp
    210                 215                 220

Ala Thr Gly Ala Val Leu Ser Ile Thr Asp Ala Lys Gly Asn Leu Gln
225                 230                 235                 240
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Arg Val Ala Tyr Asp Val Ala Gly Leu Leu Ser Gly Ser Trp Leu Thr
245 250 255

Leu Lys Asp Gly Thr Glu Gln Val Ile Val Ala Ser Leu Thr Tyr Ser
260 265 270

Ala Ala Gly Lys Lys Leu Arg Glu Glu His Gly Asn Gly Val Val Thr
275 280 285

Ser Tyr Ile Tyr Glu Pro Glu Thr Gln Arg Leu Thr Gly Ile Lys Thr
290 295 300

Glu Arg Pro Ser Gly His Val Ala Gly Ala Lys Val Leu Gln Asp Leu
305 310 315 320

Arg Tyr Thr Tyr Asp Pro Val Gly Asn Val Leu Ser Val Asn Asn Asp
325 330 335

Ala Glu Glu Thr Arg Phe Trp Arg Asn Gln Lys Val Val Pro Glu Asn
340 345 350

Thr Tyr Ile Tyr Asp Ser Leu Tyr Gln Leu Val Ser Ala Thr Gly Arg
355 360 365

Glu Met Ala Asn Ala Gly Gln Gln Gly Asn Asp Leu Pro Ser Ala Thr
370 375 380

Ala Pro Leu Pro Thr Asp Ser Ser Ala Tyr Thr Asn Tyr Thr Arg Thr
385 390 395 400

Tyr Arg Tyr Asp Arg Gly Gly Asn Leu Thr Gln Met Arg His Ser Ala
405 410 415

Pro Ala Thr Asn Asn Asn Tyr Thr Thr Asp Ile Thr Val Ser Asp Arg
420 425 430

Ser Asn Arg Ala Val Leu Ser Thr Leu Ala Glu Val Pro Ser Asp Val
435 440 445

Asp Met Leu Phe Ser Ala Gly Gly His Gln Lys His Leu Gln Pro Gly
450 455 460

Gln Ala Leu Val Trp Thr Pro Arg Gly Glu Leu Gln Lys Val Thr Pro
465 470 475 480

Val Val Arg Asp Gly Gly Ala Asp Asp Ser Glu Ser Tyr Arg Tyr Asp
485 490 495

Ala Gly Ser Gln Arg Ile Ile Lys Thr Gly Thr Arg Gln Thr Gly Asn
500 505 510

Asn Val Gln Thr Gln Arg Val Val Tyr Leu Pro Gly Leu Glu Leu Arg
515 520 525

Ile Met Ala Asn Gly Val Thr Glu Lys Glu Ser Leu Gln Val Ile Thr
530 535 540

Val Gly Glu Ala Gly Arg Ala Gln Val Arg Val Leu His Trp Glu Ile

545 550 555 560

Gly Lys Pro Asp Asp Leu Asp Glu Asp Ser Val Arg Tyr Ser Tyr Asp
565 570 575

Asn Leu Val Gly Ser Ser Gln Leu Glu Leu Asp Arg Glu Gly Tyr Leu
580 585 590

Ile Ser Glu Glu Glu Phe Tyr Pro Tyr Gly Gly Thr Ala Val Leu Thr
595 600 605

Ala Arg Ser Glu Val Glu Ala Asp Tyr Lys Thr Ile Arg Tyr Ser Gly
610 615 620

Lys Glu Arg Asp Ala Thr Gly Leu Asp Tyr Tyr Gly Tyr Arg Tyr Tyr
625 630 635 640

Gln Pro Trp Ala Gly Arg Trp Leu Ser Thr Asp Pro Ala Gly Thr Val
645 650 655

Asp Gly Leu Asn Leu Phe Arg Met Val Arg Asn Asn Pro Val Thr Leu
660 665 670

Phe Asp Ser Asn Gly Arg Ile Ser Thr Gly Gln Glu Ala Arg Arg Leu
675 680 685

Val Gly Glu Ala Phe Val His Pro Leu His Met Pro Val Phe Glu Arg
690 695 700

Ile Ser Val Glu Arg Lys Ile Ser Met Ser Val Arg Glu Ala Gly Ile
705 710 715 720

Tyr Thr Ile Ser Ala Leu Gly Glu Gly Ala Ala Ala Lys Gly His Asn
725 730 735

Ile Leu Glu Lys Thr Ile Lys Pro Gly Ser Leu Lys Ala Ile Tyr Gly
740 745 750

Asp Lys Ala Glu Ser Ile Leu Gly Leu Ala Lys Arg Ser Gly Leu Val
755 760 765

Gly Arg Val Gly Gln Trp Asp Ala Ser Gly Val Arg Gly Ile Tyr Ala
770 775 780

His Asn Arg Pro Gly Gly Glu Asp Leu Val Tyr Pro Val Ser Leu Gln
785 790 795 800

Asn Thr Ser Ala Asn Glu Ile Val Asn Ala Trp Ile Lys Phe Lys Ile
805 810 815

Ile Thr Pro Tyr Thr Gly Asp Tyr Asp Met His Asp Ile Ile Lys Phe
820 825 830

Ser Asp Gly Lys Gly His Val Pro Thr Ala Glu Ser Ser Glu Glu Arg
835 840 845

Gly Val Lys Asp Leu Ile Asn Lys Gly Val Ala Glu Val Asp Pro Ser
850 855 860

```
Arg Pro Phe Glu Tyr Thr Ala Met Asn Val Ile Arg His Gly Pro Gln
865                 870                 875                 880

Val Asn Phe Val Pro Tyr Met Trp Glu His Glu His Asp Lys Val Val
                885                 890                 895

Asn Asp Asn Gly Tyr Leu Gly Val Val Ala Ser Pro Gly Pro Phe Pro
            900                 905                 910

Val Ala Met Val His Gln Gly Glu Trp Thr Val Phe Asp Asn Ser Glu
        915                 920                 925

Glu Leu Phe Asn Phe Tyr Lys Ser Thr Asn Thr Pro Leu Pro Glu His
    930                 935                 940

Trp Ser Gln Asp Phe Met Asp Arg Gly Lys Gly Ile Val Ala Thr Pro
945                 950                 955                 960

Arg His Ala Glu Leu Leu Asp Lys Arg Arg Val Met Tyr
                965                 970
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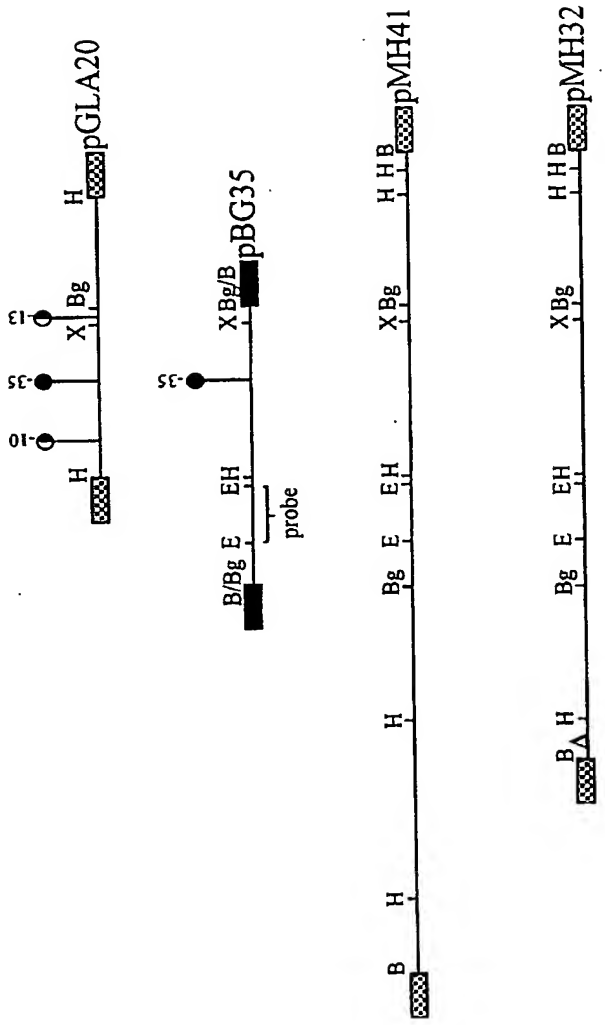
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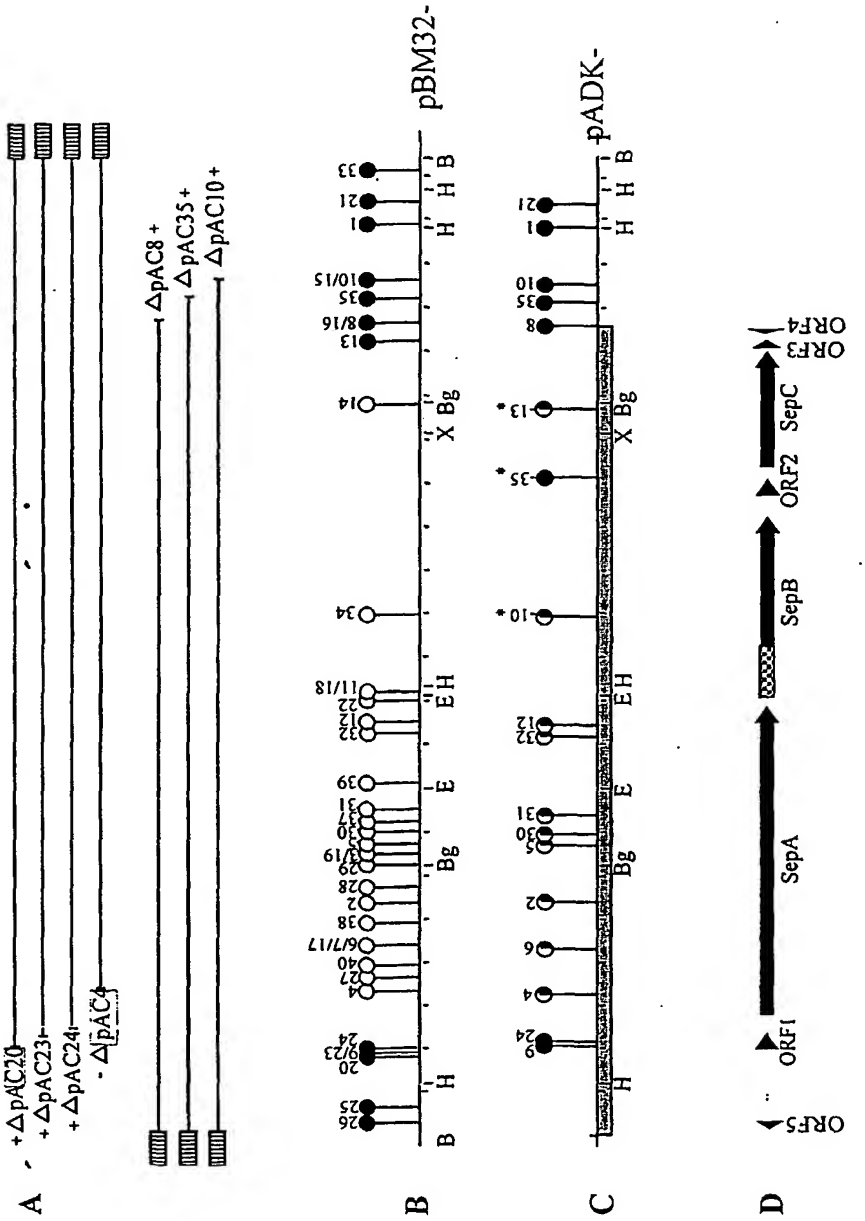
H
Bg
X
-13
-35
-10
H
pBG35
XBg/B
-35
EH
E
B/Bg
probe
pMH41
H H B
XBg
EH
Bg E
H
H
B
pMH32
H H B
XBg
EH
Bg E
H
B

FIGURE 1

FIGURE 2


A
+ ΔpAC20
+ ΔpAC23
+ ΔpAC24
- ΔpAC4
ΔpAC8 +
ΔpAC35 +
ΔpAC10 +
B
pBM32-
B H Bg E EH X H B
C
pADK-
H Bg E EH X H B
D
ORF5
ORF1
SepA
SepB
ORF2
SepC
ORF3
ORF4

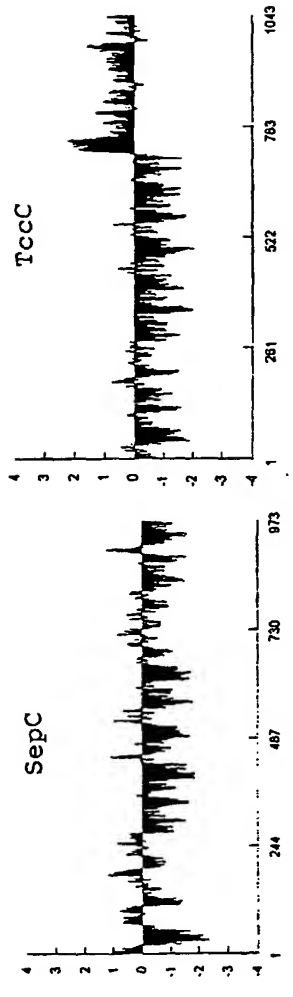

TccC
1
261
522
783
1043
4
3
2
1
0
-1
-2
-3
-4
SepC
1
244
487
730
973
4
3
2
1
0
-1
-2
-3
-4

FIGURE 3

FIGURE 4

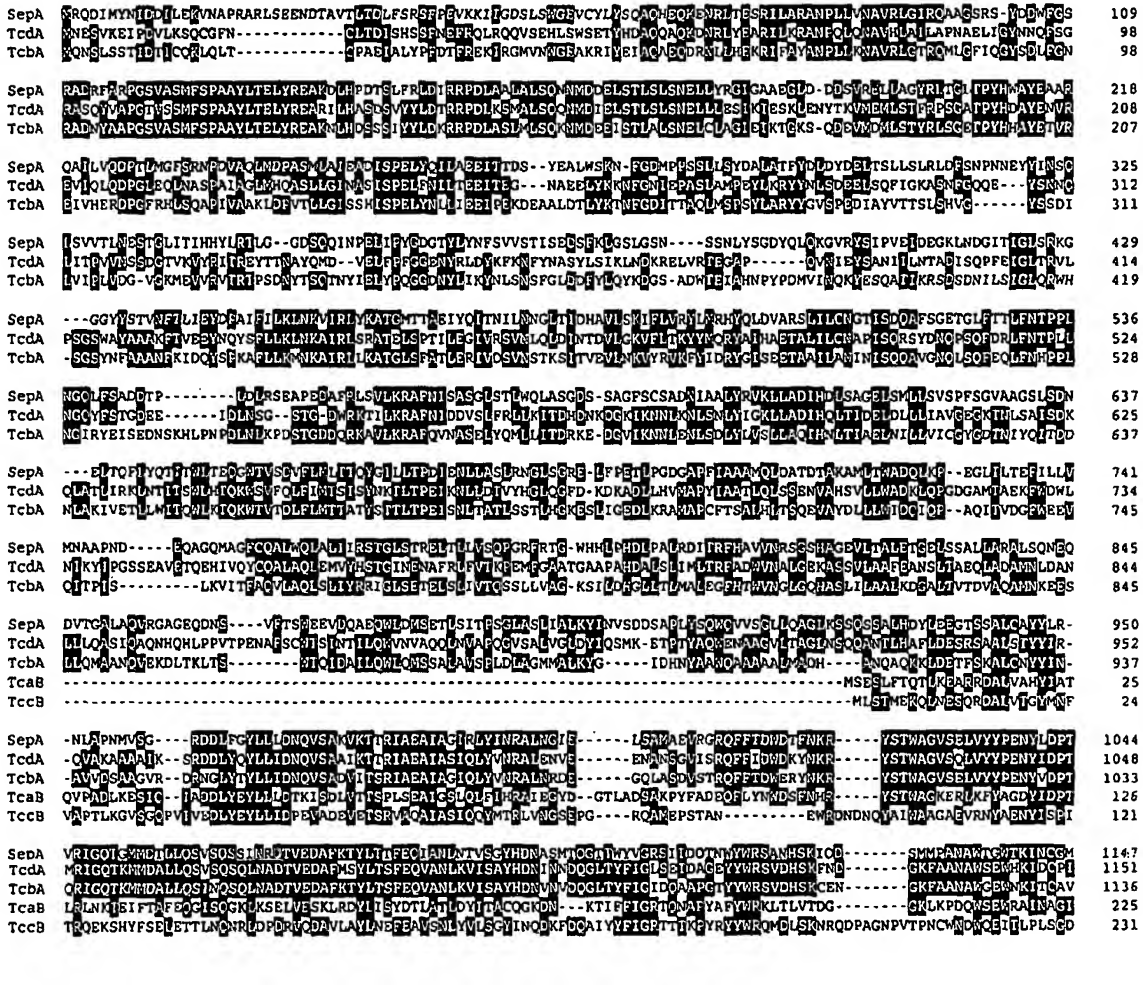


FIGURE 4 – continued

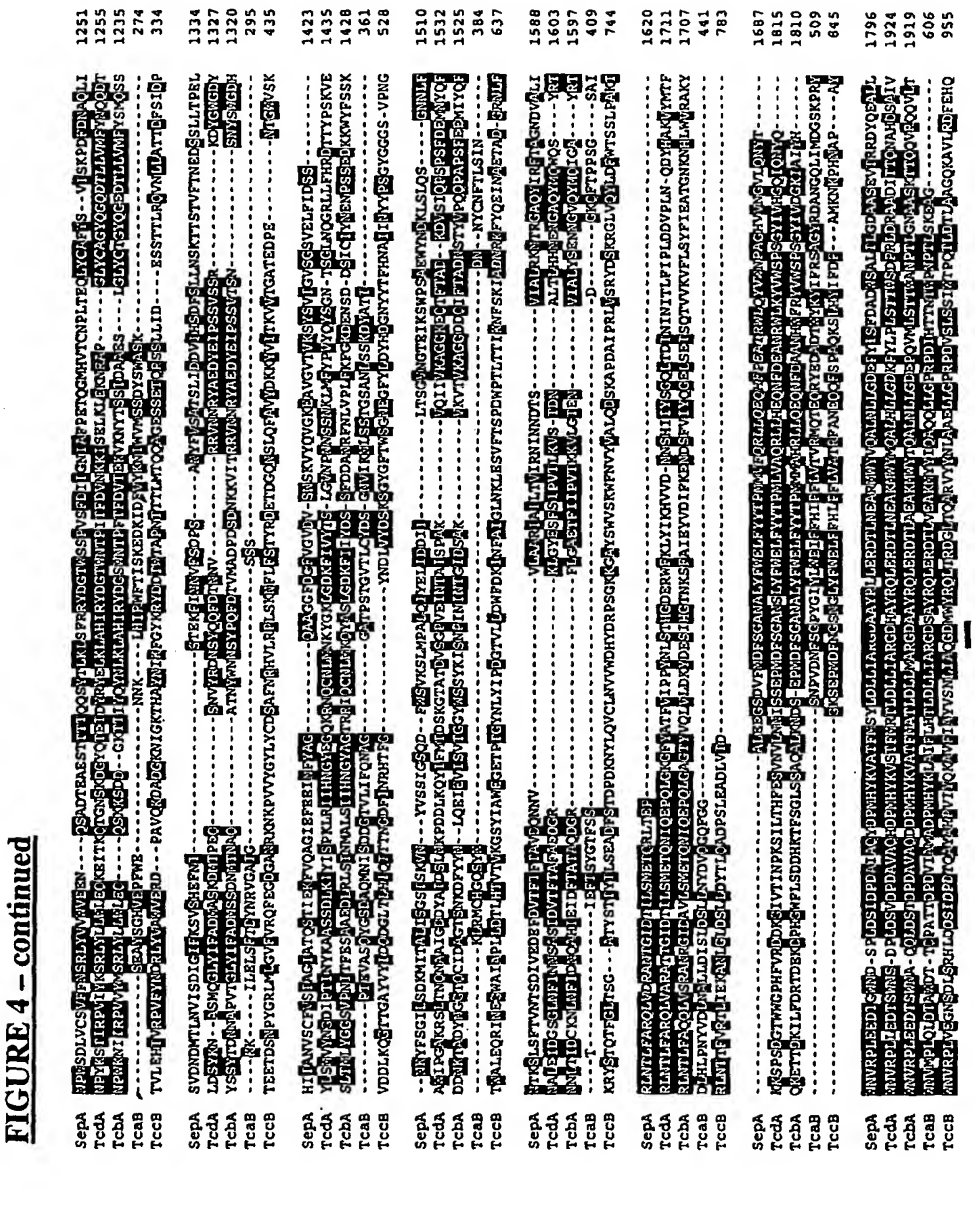
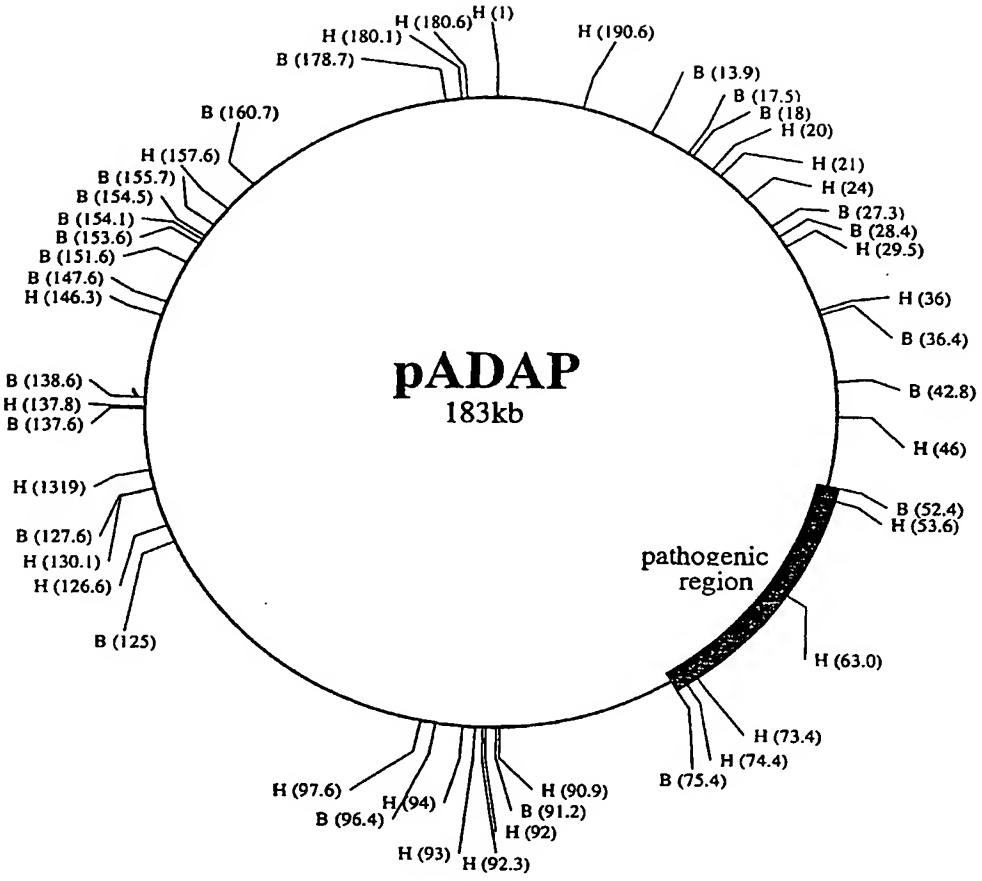


FIGURE 4 - continued

FIGURE 5

FIGURE 6


pADAP
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pathogenic region
H (1)
H (180.6)
H (180.1)
B (178.7)
H (190.6)
B (13.9)
B (17.5)
B (18)
H (20)
H (21)
H (24)
B (27.3)
B (28.4)
H (29.5)
H (36)
B (36.4)
B (42.8)
H (46)
B (52.4)
H (53.6)
H (63.0)
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H (74.4)
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H (92)
H (92.3)
H (93)
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H (130.1)
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B (151.6)
B (153.6)
B (154.1)
B (154.5)
B (155.7)
H (157.6)
B (160.7)

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Glare, Travis T
Hurst, Mark R H
Jackson, Trevor A

(ii) TITLE OF INVENTION: Insecticidal nucleotide sequences

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: A J Park & Son
(B) STREET: Huddart Parker Building, Post Office Square
(C) CITY: Wellington
(D) COUNTRY: New Zealand

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18937 nucleotides
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: Linear

(A) LENGTH: 5118 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: DNA

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ggatccgagt gaaggaatca tcggccgctt tatacgtttc agggtgaata cggttggccg 60

caacgtggca atggatgttg tttgtgtcgg tatgaatcgc cgcaacgtac tggtgttctg 120

acatacccag tgccgataaa ctgtgacgaa cactatcaaa gatgtgttcc gtcgacctga 180

aagccaggat ttatttttac accaatggtt gggtgggctt cctttctgaa ctggtgcatc 240

atttagccgg catcatcaaa agatgcatgg aaatacaaat atcatattta cagacaccca 300

agttgatgac ctgctccgtg agttgaaatg ccgacggggg aaatcagcag ccttttcaac 360

tcatggagca gggggaaatc aatcctcaat aacccgcatt ggatatcctg ccagtgtgca 420

tttaaccttt ttagtgtgtt tccttaatat cccaatcgtt gaatcgctac atacggcaga 480

cattagtatc tcacttatca tcaaagtaat atcacaccga gaatgctaat ttcatgatat 540

gaaaacgttc cattaataaa ttttcagaaa cctaacacgg catttttatg ctgatcagtg 600

aattgattgt ttctgaaaaa attaattgca cctctgccac ttatcagata aaaacacccc 660

atgcggtaag ttttttattt tttattaatg attttattaa tgattttatt aatgatttta 720

ttaatgattt tattaatgat tttactatag atgaatgtta acatgggtga taatttactt 780

tactcaattt aattgttggt atgaccatgt tttagatgag tggcacggat tcattattgt 840

aaaaaaagta tctaaaacct ttagcagcaa tcctacttga ggatgacctc gacaggactt 900

gattattgcc atttttttacg aaggaagatg acgggtgata aataataaaa aaaacaaaag 960

tatagcctta ggtatcgccg attacatcca gtaacactta ttgacttttt tttacttcta 1020

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
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PCT

(10) International Publication Number
WO 01/16305 A3

(51) International Patent Classification[7]: C12N 15/31, 15/70, 15/82, C07K 14/24, C12Q 1/68, A01N 63/02, A01H 5/00

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(22) International Filing Date:
4 September 2000 (04.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
337610 2 September 1999 (02.09.1999) NZ

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(75) Inventors/Applicants *(for US only)*: **GLARE, Travis, Robert** [AU/NZ]; 38 Whincorps Road, Halswell, Christchurch 8003 (NZ). **HURST, Mark, Robin, Holmes** [NZ/NZ]; 148 Hendersons Road, Hoon Hay, Christchurch 8002 (NZ). **JACKSON, Trevor, Anthony** [NZ/NZ]; 407 Halswell Road, Halswell, Christchurch 8003 (NZ).

(74) Agent: **WILSON, Kathryn, S.**; all of Level 12,, KPMG Center,, 85 Alexandra Street, Private Bag 3140, Hamilton (NZ).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
10 January 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/16305 A3

(54) Title: NUCLEOTIDE SEQUENCES ENCODING AN INSECTIDAL PROTEIN COMPLEX FROM SERRATIA

(57) Abstract: The present invention concerns novel nucleotide sequences encoding proteins from the Enterobacteriaceae, *Serratia entomophila* and *Serratia proteamaculans*, and the use of said nucleotide sequences and proteins for inherent insecticidal and potentially metazoacidal properties. The invention relates to an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with the nucleic acid molecule under standard hybridisation conditions. The nucleotide sequences include a pathogenicity-encoding region cloned from bacteria *Serratia entomophilia* and *S. proteamaculans*. The region contain pathogenic determinants of a disease that affect the grass grub, *Costelytra zealandica Coleoptera: Scarabaeidae*, an important insect pasture pest in New Zealand. The proteins encoded by determined genes may be used for insect control whether as an inundative pesticide, within baits or expressed in other organisms such as plants or microbes.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NZ 00/00174

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C12N15/70 C12N15/82 C07K14/24 C12Q1/68
A01N63/02 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, STRAND, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JACKSON T A ET AL: "PATHOGEN TO PRODUCT DEVELOPMENT OF SERRATIA-ENTOMOPHILA ENTEROBACTERIACEAE AS A COMMERCIAL BIOLOGICAL CONTROL AGENT FOR NEW ZEALAND GRASS GRUB COSTELYTRA-ZEALANDICA" JACKSON, T. A. AND T. R. GLARE (ED.). USE OF PATHOGENS IN SCARAB PEST, 1992, pages 191-198, XP000997900 0-946707-35-9. 1992 the whole document --- -/--	32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2001	06/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRKOVIC STEVE ET AL: "Genes Essential for Amber Diseae in Grass Grubs Are Located on the Large Plasmid Found in Serratia entomophila and Serratia proteamaculans." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 61, no. 6, 1995, pages 2218-2223, XP000994573 ISSN: 0099-2240 cited in the application the whole document	
A	GLARE TRAVIS R ET AL: "Plasmid transfer among several members of the family Enterobacteriaceae increases the number of species capable of causing experimental amber disease in grass grub." FEMS MICROBIOLOGY LETTERS, vol. 139, no. 2-3, 1996, pages 117-120, XP000998482 ISSN: 0378-1097 cited in the application the whole document	
A	WO 99 42589 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); KRAMER VANCE CARY) 26 August 1999 (1999-08-26) the whole document	
A	WO 98 08932 A (DOW AGROSCIENCES LLC ;WISCONSIN ALUMNI RES FOUND (US)) 5 March 1998 (1998-03-05) the whole document	
A	WO 98 08388 A (MORGAN JAMES ALUN WYNNE ;JARRETT PAUL (GB); ELLIS DEBORAH JUNE (GB) 5 March 1998 (1998-03-05) the whole document	
A	WO 97 17432 A (WISCONSIN ALUMNI RES FOUND) 15 May 1997 (1997-05-15) the whole document	
A	BOWEN D ET AL: "INSECTICIDAL TOXINS FROM THE BACTERIUM Photorhabdus liminescens" SCIENCE,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,,US, vol. 280, 26 June 1998 (1998-06-26), pages 2129-2132, XP002115650 ISSN: 0036-8075 cited in the application	

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NUNEZ-VALDEZ M E ET AL: "The amb2 locus from Serratia entomophila confers anti-feeding effect on larvae of Costelytra zealandica (Coleoptera: Scarabaeidae)" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 172, no. 1, 12 June 1996 (1996-06-12), pages 75-79, XP004042712 ISSN: 0378-1119 cited in the application	
P,X	HURST MARK R H ET AL: "Plasmid-located pathogenicity determinants of Serratia entomophila, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of Photorhabdus luminescens." JOURNAL OF BACTERIOLOGY, vol. 182, no. 18, September 2000 (2000-09), pages 5127-5138, XP002166799 ISSN: 0021-9193 the whole document	1-4, 9-16, 21-27, 31,41

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17

Present claim 17 relates to a ligand defined by reference to a desirable characteristic or property, namely binding to the polypeptide of claim 15.

The claim covers all products having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for such products.
In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved.
Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NZ 00/00174

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9942589 A	26-08-1999	AU 3028699 A	06-09-1999
		EP 1054972 A	29-11-2000
WO 9808932 A	05-03-1998	AU 729228 B	25-01-2001
		AU 1050997 A	29-05-1997
		AU 2829997 A	19-03-1998
		BR 9606889 A	28-10-1997
		BR 9711441 A	24-10-2000
		CA 2209659 A	15-05-1997
		EP 0797659 A	01-10-1997
		EP 0970185 A	12-01-2000
		HU 9900768 A	28-06-1999
		JP 2000515024 T	14-11-2000
		PL 321212 A	24-11-1997
		PL 332033 A	16-08-1999
		SK 24699 A	10-04-2000
		SK 93197 A	06-05-1998
		TR 9901126 T	21-07-1999
		WO 9717432 A	15-05-1997
WO 9808388 A	05-03-1998	AU 4024997 A	19-03-1998
		BR 9711285 A	17-08-1999
		CN 1233938 A	03-11-1999
		EP 0923295 A	23-06-1999
		TR 9900435 T	21-06-1999
		ZA 9707373 A	15-02-1999
WO 9717432 A	15-05-1997	AU 729228 B	25-01-2001
		AU 1050997 A	29-05-1997
		BR 9606889 A	28-10-1997
		CA 2209659 A	15-05-1997
		EP 0797659 A	01-10-1997
		HU 9900768 A	28-06-1999
		PL 321212 A	24-11-1997
		PL 332033 A	16-08-1999
		SK 93197 A	06-05-1998
		AU 2829997 A	19-03-1998
		BR 9711441 A	24-10-2000
		EP 0970185 A	12-01-2000
		JP 2000515024 T	14-11-2000
		SK 24699 A	10-04-2000
		TR 9901126 T	21-07-1999
		WO 9808932 A	05-03-1998

common function.

The present applicant has now found that three regions of the pADAP plasmid are required for full insecticidal function. Sequence analysis of these three regions has shown that the present applicant has isolated and identified a novel toxin from *Serratia* species that belongs to a new family of insecticidal toxins. It is broadly to this toxin that the present invention is directed.

DISCLOSURE OF INVENTION

According to a first aspect of the present invention, there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 1 which encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with said nucleic acid molecule under standard hybridisation conditions.

The invention also provides an isolated nucleic acid molecule comprising the nucleotide sequence 1995-18937 of SEQ ID NO: 1 which encodes an insecticidal protein complex, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with said nucleic acid molecule under standard hybridisation conditions.

The invention also provides an isolated nucleic acid molecule comprising one or more of the nucleotide sequences 2411-9547, 9589-13883 or 14546-17467 of SEQ ID NO: 1 which encode insecticidal proteins, or a functional fragment, neutral mutation, or homolog thereof capable of hybridising with said nucleic acid molecule under standard hybridisation conditions.

Preferably the nucleic acid molecule comprises all of nucleotide sequences 2411-9547, 9598-13884 and 14546-17467 of SEQ ID NO: 1.

The invention further relates to an isolated nucleic acid molecule comprising a sequence of SEQ ID NO: 1, nucleotides 1955-18937 of SEQ ID NO: 1 or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein. For example, the at least one further nucleotide sequence may be the nucleotide sequence which codes for the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins and so forth.

The nucleic acid molecule may comprise DNA, cDNA or RNA.

Preferably said fragment, neutral mutation or homolog thereof is capable of hybridising to said nucleic acid molecule under stringent hybridisation conditions.

The invention further relates to nucleic acid molecules which hybridise to the nucleotide sequence of SEQ ID NO: 1, or nucleotides 1955-18937, 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1 if there is at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity between the sequences.

The nucleic acid molecule may be isolated from *Serratia entomophila* or *Serratia proteamaculans* strains.

Also provided by the present invention are recombinant expression vectors containing the nucleic acid molecule of the invention and hosts transformed with the vector of the invention capable of expressing a polypeptide of the invention.

The vector may be selected from any suitable natural or artificial plasmid/vector. For example, pUC 19 (Yannish-Perron et al. 1995), pProEX HT (GibcoBRL, Gaithersburg, MD, USA), pBR322 (Bolivar et al. 1977), pACYC184 (Chang et al. 1978), pLAFR3 (Staskowicz et al. 1987), and so forth.

THE CLAIMS DEFINING THE INVENTION ARE:

1. A purified and isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 1 that encodes at least one of:

   (i) an insecticidal protein complex, or

   (ii) a functional fragment of said complex, or

   (iii) a neutral mutation of said complex, or

   (iv) a homolog of said complex,

   each of which are capable of hybridising with said nucleic acid molecule under standard hybridisation conditions.

2. A purified and isolated nucleic acid molecule as claimed in Claim 1comprising the nucleotide sequence 1995-18937 of SEQ ID NO: 1.

3. A purified and isolated nucleic acid molecule as claimed in Claim 1 comprising one or more of the nucleotide sequences 2411-9547, 9589-13883 or 14546-17467 of SEQ ID NO: 1.

4. A purified and isolated nucleic acid molecule as claimed in Claim 3 comprising all of nucleotide sequences 2411-9547, 9598-13884 and 14546-17467 of SEQ ID NO: 1.

5. A purified and isolated nucleic acid molecule as claimed in Claim 1 comprising a sequence of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

6. A purified and isolated nucleic acid molecule as claimed in Claim 2 comprising nucleotides 1955-18937 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

7. A purified and isolated nucleic acid molecule as claimed in Claim 3 comprising a sequence of SEQ ID NO: 1,or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which encode an insecticidal protein.

8. A purified and isolated nucleic acid molecule as claimed in any one of claims 4 through 6 wherein the said nucleotide sequence includes the nucleotide sequence which codes for at least one of the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins.

9. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein nucleic acid molecule may comprise DNA, cDNA or RNA.

10. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein the nucleic acid molecules said fragment, neutral mutation or homolog thereof capable of hybridising to said nucleic acid molecule, hybridise to the nucleotide sequence of SEQ ID NO: 1, or nucleotides 1955-18937, 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1 if there is at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity between the sequences.

11. A purified and isolated nucleic acid molecule as claimed in claim 1 wherein the nucleic acid molecule may be isolated from *Serratia entomophila* or *Serratia proteamaculans* strains of bacteria.

12. A recombinant expression vector(s) containing the nucleic acid molecule as claimed in Claim 1 and host transformed with the vector expressing a polypeptide.

13. A recombinant expression vector(s) as claimed in claim 11 wherein the vector is selectable from any suitable natural or artificial plasmid/vector.

14. A recombinant expression vector(s) as claimed in claim 13 wherein said suitable natural or

artificial plasmid/vector, including, pUC 19 (Yannish-Perron et al. 1995), pProEX HT (GibcoBRL, Gaithersburg, MD, USA), pBR322 (Bolivar et al. 1977), pACYC184 (Chang et al. 1978), pLAFR3 (Staskowicz et al. 1987).

15. A polypeptide resulting from the transformation or transfection of a host cell with a recombinant expression vector as claimed in any one of Claims 12 through 14.

16. A method of producing a polypeptide of claim 15 comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with said vector as defined above to express the encoded polypeptide or peptide; and

(b) recovering the expressed polypeptide or peptide.

17. A ligand that binds to a polypeptide of Claim 15.

18. A ligand as claimed in claim 17 wherein the ligand is an antibody or antibody binding fragment.

19. Probes and primers comprising a fragment of the nucleic acid molecule as claimed in Claim 1 wherein said fragment is hybridisable under stringent conditions to a native insecticidal gene sequence.

20. Probes and primers comprising a fragment of the nucleic acid molecule as claimed in claim 19 wherein said probes and primers enable the structure and function of the gene to be determined and homologs of the gene to be obtained from bacteria other than *Serratia* sp.

21. A polypeptide as claimed in Claim 15 wherein the polypeptide has insecticidal activity encoded by the nucleic acid molecule of claim 1, or a functional fragment, neutral mutation or homolog thereof.

22. A polypeptide having insecticidal activity as claimed in claim 21 wherein the polypeptide